NOV 19 1943

VOLUME XIX, No. 6 WHOLE No. 115

NOVEMBER, 1943

Medical Library

THE AMERICAN JOURNAL OF PATHOLOGY

Official Publication of
The American Association of Pathologists and Bacteriologists

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EAST UNIVERSITY AVENUE, ANN ARBOR, MICHIGAN
Issued Bimonthly Annual Subscription in U. S. A. $8.00

Entered as second class matter, January 23, 1941, at the Post Office at Ann Arbor, Michigan, under the Act of March 3, 1879

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THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME XIX NOVEMBER, 1943 NUMBER 6

HYPERPLASIA OF THE PULMONARY ALVEOLAR EPITHELIUM IN DISEASE*

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The pulmonary alveoli develop as saccular outgrowths of the bronchioles. In the early part of fetal life the lung has a glandular appearance and the alveoli are lined by a continuous epithelial layer. But in the latter part of the intra-uterine period the capillary bed develops extensively, and numerous capillaries break through the epithelial lining, leaving the epithelium as isolated rounded cells. A clear account of the development of the alveoli was given by Ham and Baldwin,[1] who found that they are lined chiefly by naked capillaries from late fetal life onward and that only a few rounded epithelial cells persist. In the postnatal lung occasional epithelial cells may be demonstrated in the niches between capillaries (Clara [2]).

It seems well established that in the postnatal lung the alveolar walls are largely bare of epithelium but that occasional epithelial cells may be found. This disappearance of the alveolar lining is presumably a functional alteration favoring a more rapid interchange of gases between the blood and the alveolar air, and it may be compared to the disappearance of the endothelial lining of the glomerular capillaries which promotes filtration through their walls.

Although lining epithelial cells are inconspicuous in the normal lung, there are numerous pulmonary diseases in which they become prominent and form a continuous epithelial layer. The various circumstances under which hyperplasia of alveolar epithelium occurs will now be described.

Chronic Passive Congestion. In long-standing chronic passive congestion due to old mitral or aortic valvular defects the lungs usually show a moderate increase of consistency throughout, and often there are firm consolidated areas suggesting pneumonia on macroscopic examination. Microscopic sections from nonconsolidated portions show a little increase in the thickness of the interalveolar septa; and a few

* Received for publication, February 3, 1943.

epithelial cells, single or in small groups, are readily found (Fig. 1). In the consolidated areas the interalveolar septa are very thick and the alveoli are lined by a continuous layer of dark cubical epithelium (Fig. 2). The thick interalveolar septa show numerous fibrocytes and a loose edematous fibrillar connective tissue. The capillaries are reduced in number and so far from the surface that it is unlikely that any considerable interchange of gases can occur. Parker and Weiss [3] described this lesion and attributed the thick septa to chronic edema. Thickening of the septa and immobilization of the tissue precede the epithelization of the alveoli. A study of transition stages shows that the new epithelial lining arises by multiplication of pre-existent alveolar epithelial cells and not as an ingrowth of bronchial epithelium.

Lipoid Pneumonia. In lipoid pneumonia the alveoli that contain fat become filled with macrophages and are largely nonfunctioning because very little air can gain entrance. In such alveoli several observers have noted epithelization. The alveolar epithelium may be present in patches or it may form a continuous layer (Fig. 3). A study of transition stages indicates hyperplasia of persistent alveolar epithelium as in chronic passive congestion. The epithelial cells are often desquamated, but they apparently do not act as phagocytes. As in chronic passive congestion, epithelization is preceded by thickening of the interalveolar septa and loss of function.

Chronic Interstitial Pneumonia. In unresolved pneumonia there are often areas of chronic interstitial pneumonia, and in such lesions there are frequently foci with marked epithelization of the alveoli. Oberndorfer [4] described such changes in a case of hemorrhagic pneumonia.

As an example of epithelization in chronic interstitial pneumonia the following case is cited: A man, 67 years old, died of pneumonia of unknown duration. The pathologist, Dr. J. F. Noble, found large irregular areas of consolidation of fibrous consistency in each lung. Upon microscopic examination the consolidated areas were found to be chiefly due to interstitial pneumonia. Within and around the solidified areas many of the alveoli were lined by cubical or columnar epithelium, giving the tissue a glandular appearance (Fig. 4). Some of the columnar cells were filled with mucin but no ciliated cells were seen. The presence of cylindrical cells filled with mucin suggests that bronchial epithelium has grown into the alveoli, but numerous transition stages indicate that the epithelium develops from small cells that were already present on the alveolar wall. The first stage in epithelization is the formation of a discontinuous epithelial layer (Fig. 5), which then increases to form a solid cubical or columnar layer. Many of the epithelial cells are desquamated into the alveoli.

Grumbach [5] studied chronic interstitial pneumonia in guinea-pigs, which he attributed to injections of a diphtheroid bacillus obtained from a lymph node of a patient with Hodgkin's disease. One may doubt whether the pneumonia was due to the organism injected, but a diffuse alveolar epithelization developed which Cowdry [6] considered similar to that in the lungs of jaagsiekte in sheep.

Epithelization of the alveoli is of common occurrence in diseased lungs. It is often found adjacent to the walls of old empyema cavities and old pleuritic adhesions as well as in all forms of interstitial pneumonia. The basic disturbance responsible for the formation of alveolar epithelium seems to be a loss of respiratory function due to thickening of the interalveolar septa or filling of the alveoli with foreign material.

Tar and Tar Derivatives. Simonds and Curtis [7] injected tar, dissolved in heavy liquid petrolatum, intravenously into rabbits, and obtained marked alveolar epithelization about necrotic areas in the lungs.

Grady and Stewart [8] induced pulmonary tumors in strain A mice by subcutaneous injection of 1:2:5:6-dibenzanthracene or methylcholanthrene. Multiple tumors began to develop about 5 weeks after the injection. The tumors were all of alveolar origin, and they began as a layer of cells lining the alveoli. The lining epithelium then continued to grow to form a carcinoma. The authors pointed out the similarity of the lesion to jaagsiekte in sheep.

Jaagsiekte. Jaagsiekte is a serious endemic infectious disease in sheep prevalent in South Africa. A similar disease in sheep in Iceland was described by Dungal.[9] Among the prominent symptoms are dyspnea, easy fatigability and a watery discharge from the lungs. The lungs are extensively consolidated. Cowdry and Marsh [10] have described the histologic changes. The alveoli are all lined by cubical or columnar epithelium, giving the lung a glandular appearance. The structure suggests carcinoma, but there are no metastases and the interalveolar septa are intact. The etiology of jaagsiekte is unknown, but its infectious nature and the peculiar proliferative reaction limited to alveolar epithelium suggests a virus.

DIFFUSE EPITHELIAL HYPERPLASIA IN MAN

Bonne,[11] in 1939, described an unusual pulmonary lesion in a Chinese male, 30 years of age. The duration of the disease was about 9 months. It began with a fever of about 4 weeks' duration followed by a cough which persisted. The sputum contained mucus but no blood. In the later months there was dyspnea and emaciation, and roentgenograms showed a massive consolidation of the left lung. The blood picture was normal. At autopsy the lungs weighed 2700 gm.,

and were almost completely solid and homogeneous. Only the right apex was uninvolved. The appearance suggested lobar pneumonia with hepatization but the lungs were a little firmer. Microscopically all of the alveoli were lined by cubical or columnar epithelium. Bonne at first considered the growth to be a carcinoma but, since there were no metastases and the interalveolar septa were intact, he finally interpreted it as adenomatosis. He pointed out the striking resemblance to jaagsiekte in sheep.

Report of Case

A case similar to Bonne's [11] came under my observation. The patient, a male, 63 years of age, was admitted to the University Hospital on March 5, 1942, on the service of Dr. C. J. Watson, complaining of dyspnea on exertion, productive cough, weakness and loss of weight. His illness began about 2 years before admission with these symptoms. He had dyspnea on slight exertion, and he produced an abundant, clear, frothy sputum. He had lost about 25 lbs. He had no fever, chills or night sweats. Hemoglobin was 14 gm. per cent; leukocytes, 12,000, with 72 per cent neutrophils and 24 per cent lymphocytes. The urine was normal. The Wassermann reaction was negative. Physical examination revealed consolidation of the left lower and right middle lobes. After introduction of lipiodol the small bronchi were of unusual appearance in that they showed a diffuse narrowing. The tentative clinical diagnosis was carcinoma of the lung.

The patient was finally dismissed from the hospital, and he died at home on November 16, 1942. The family physician removed parts of the lungs and sent them to the University. He made no observations as to metastases.

The specimens submitted were the consolidated lobes of each lung. These had the appearance of lobar pneumonia in that there was a diffuse consolidation. Microscopic examination showed changes entirely similar to those described by Bonne.[11] Every alveolus was lined by cubical or columnar epithelium (Figs. 6 and 7). The interalveolar septa were moderately increased in thickness but this was due chiefly to the epithelial layers and not to leukocytic infiltration and fibrosis as in interstitial pneumonia. The septa were never destroyed by epithelial invasion as one would expect if the growth were a carcinoma.

The lesion is clearly not an interstitial pneumonia, and epithelization of the alveoli is not due to thickening of the septa or to foreign material in the alveoli. There is presumably some direct stimulation of the alveolar lining cells. The epithelial layer separates the capillaries from the alveolar air and greatly impedes the interchange of gases. This is presumably the cause of the dyspnea.

ADENOMAS AND CARCINOMAS ARISING FROM THE ALVEOLAR EPITHELIUM

There is a widespread opinion that all carcinomas of the lungs arise from the bronchi, yet there are a few reported cases with strong evidence of an alveolar origin.

Helly,[12] in 1907, described multiple adenomas of the lung in a woman, 43 years old. Richardson,[13] in 1940, reported numerous firm nodules, 2 to 10 mm. in diameter, in the lungs of a woman, 73 years old. The tumors were sharply circumscribed and did not connect with bronchi. Microscopically the growths were composed of closely set alveoli lined by tall columnar epithelium which formed mucin. The interpretation was multiple adenomas.

Weissmann,[14] in 1935, described two instances of alveolar tumors. (a) A male, 73 years old, with numerous gray nodules in both lower lobes. The interalveolar septa appeared to be normal and were lined by tall columnar epithelium. (b) A woman, 65 years old, showed numerous nodules throughout both lungs with metastases in the bronchial nodes. Microscopically the alveolar structure was retained and the alveoli were lined by tall columnar epithelium.

Sweany,[15] in 1935, described a lung from a woman, 67 years old, which had the macroscopic appearance of lobar pneumonia. Microscopically there was an alveolar structure with alveoli lined by a single layer of epithelium. There was a metastasis in one bronchial lymph node.

Neubuerger,[16] in 1941, published the report of a male patient, 39 years of age, with multiple yellowish nodules, 2 to 10 mm. in diameter, throughout both lungs. There were metastases in the mediastinal and periaortic lymph nodes, the liver and the kidneys. Microscopically the alveoli were lined by tumor cells but the interalveolar septa were intact.

The human pulmonary tumors reported as multiple adenoma or carcinoma have a microscopic structure similar to that of my case and the one reported by Bonne.[11] The alveoli are lined by epithelial cells and the interalveolar septa are intact. In a few instances metastases have been found. There is no good reason to doubt that hyperplasia of the alveolar epithelium may give rise to localized or diffuse adenomatous growths which may form metastases.

Discussion

In the postnatal lung the alveolar walls are formed almost entirely by the capillaries, but a few epithelial cells persist in the niches between capillaries. In any disease which brings about marked thickening of the interalveolar septa, with displacement of the capillaries away from the surface and consequent loss of respiratory function, the alveolar epithelium may undergo hyperplasia to form a continuous epithelial lining. The cells are either cubical or columnar and some of them may secrete mucin. There is convincing evidence that the

epithelium forms locally and does not grow in from the bronchi. In chronic passive congestion and in interstitial pneumonia, epithelization of the alveoli follows thickening of the interalveolar septa.

Mild irritation of the alveolar walls from foreign bodies may bring about epithelization, as in lipoid pneumonia.

In jaagsiekte of sheep widespread epithelization of the alveoli occurs. In this infectious disease the etiology is unknown but the microscopic features suggest direct irritation of the cells by a virus which attacks them.

In man there are now two examples of widespread epithelization similar to jaagsiekte in sheep (Bonne,[11] Bell). These may be regarded as diffuse epithelial hyperplasia due to an unknown irritant. In the absence of metastases and destructive infiltrative growth, the process in these cases can hardly be interpreted as neoplasm, but Sweany's [15] report suggests a neoplastic nature.

Summary

A case of extensive diffuse epithelization of the alveoli of the human lungs is reported which is similar to the one reported by Bonne.[11]

Epithelization of the alveoli is often seen in chronic passive congestion and interstitial pneumonia, apparently as a result of thickening of the interalveolar septa.

Foreign material in the alveoli also causes epithelization, as in lipoid pneumonia.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

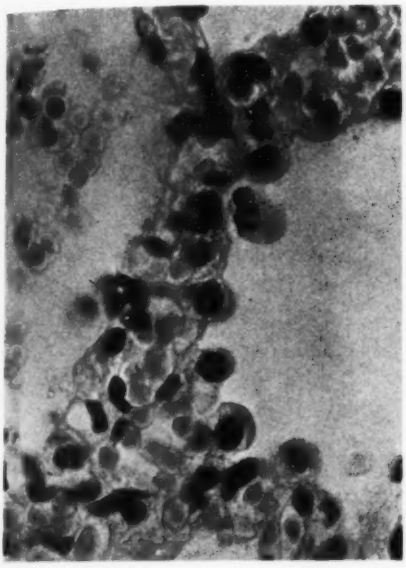
PLATE 109

FIG. 1. Chronic passive congestion from mitral stenosis and aortic insufficiency. A nonconsolidated area of the lung shows several rounded alveolar epithelial cells on the right side of the interalveolar septum. The greater part of the septal walls is bare. Hematoxylin and eosin stain. × 350.

FIG. 2. Chronic passive congestion from an old rheumatic defect of the aortic valve. Thick interalveolar septum from a consolidated area. The alveoli are lined by dark cubical epithelium, and the few small capillaries are at some distance from the surface. Hematoxylin and eosin stain. × 250.

FIG. 3. Lipoid pneumonia showing macrophages filled with fat in the alveoli, and a layer of alveolar epithelial cells on the wall of the septum. Hematoxylin and eosin stain. × 250.

FIG. 4. Chronic interstitial pneumonia. The persisting alveoli are lined chiefly by tall columnar cells filled with mucin. Hematoxylin and eosin stain. × 80.



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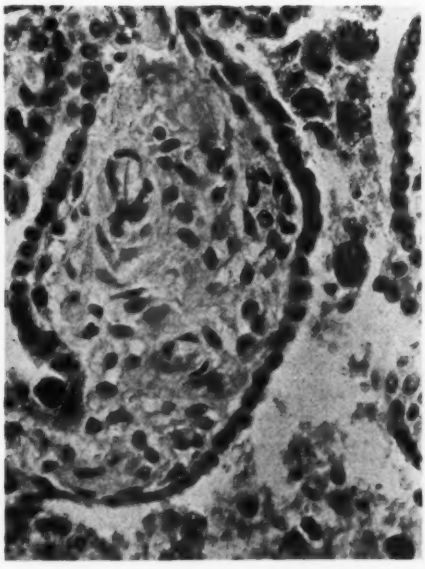

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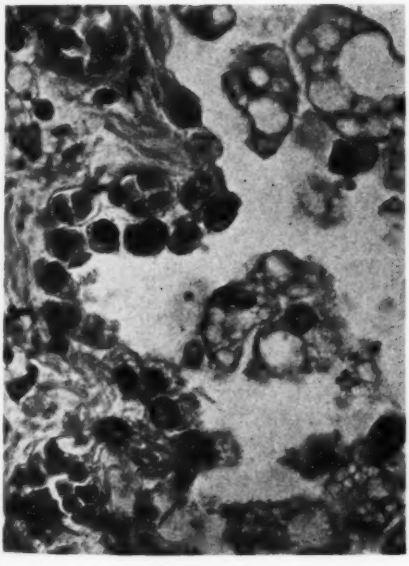

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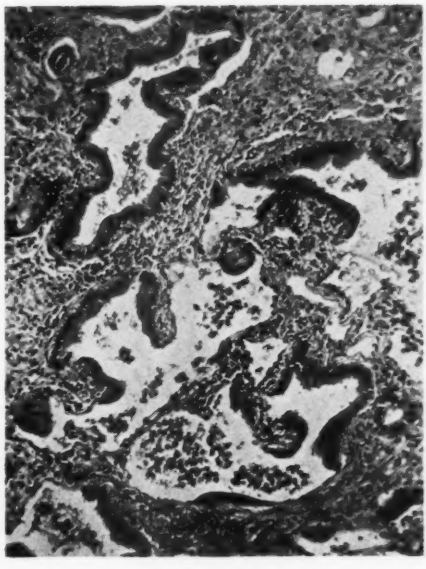


4

Bell

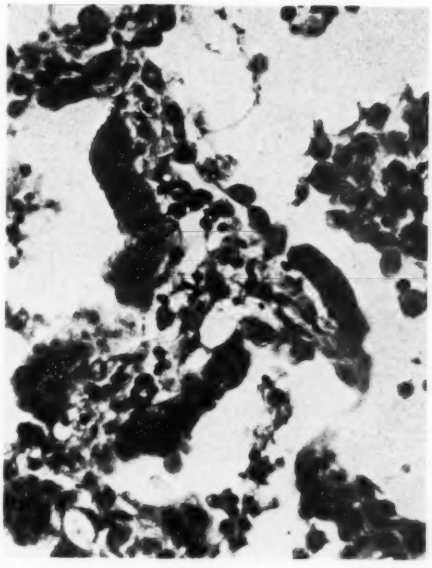
Hyperplasia of Pulmonary Alveolar Epithelium

PLATE 110

FIG. 5. Chronic interstitial pneumonia. From the same lung as Figure 4. The alveolar epithelium forms a thin discontinuous layer on the upper right side of the septum and a solid epithelial layer in other places. Some desquamated epithelial cells are seen in the alveoli. Hematoxylin and eosin stain. × 250.

FIG. 6. Area of consolidated lung from the author's case. The alveoli are all lined by cubical or columnar epithelium. Hematoxylin and eosin stain. × 60.

FIG. 7. Same area as in Figure 6 under higher magnification. The thickening of the septa is due chiefly to the epithelial layers. Hematoxylin and eosin stain. × 100.



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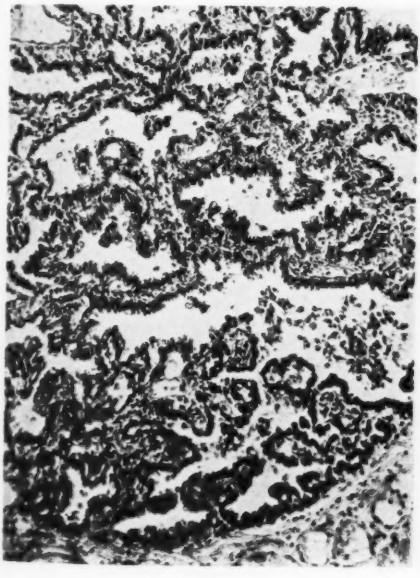

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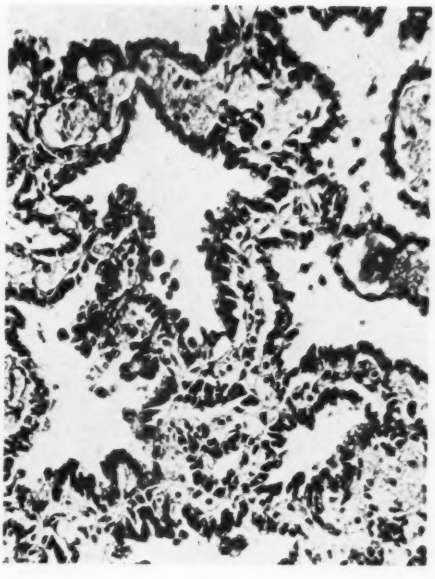

7

Bell

Hyperplasia of Pulmonary Alveolar Epithelium

THE PULMONARY ALVEOLAR LINING UNDER VARIOUS PATHOLOGIC CONDITIONS IN MAN AND ANIMALS*

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The presence of lining cells in the pulmonary alveoli in various pathologic conditions has often been mentioned in papers and textbooks. In addition, proliferation of these alveolar lining cells has been produced experimentally. To our knowledge, however, El Gazayerli [1] is the only author who ever attempted to collect and describe such pathologic material systematically. This lack of organized studies perhaps explains the absence of a special chapter on this subject in most modern American textbooks of pathology. Bell [2] alone discusses the matter briefly. Contemporary pathologists (Bell,[2] Boyd,[3] Karsner,[4] MacCallum,[5] Smith and Gault,[6] Delafield and Prudden [7]) apparently accept the presence normally of alveolar epithelium, which proliferates under various stimulations. On the other hand, anatomists (Maximow and Bloom,[8] Cowdry,[9] Miller [10]) disagree as to the structure of the alveolar wall and as to the existence of an alveolar lining. Anatomists, however, do not treat thoroughly the subject of alveolar cell proliferation because it is a phenomenon found in pathologic conditions. Our interest in the subject became aroused during an investigation of lung tumors believed to take origin from the pulmonary alveoli. As a result, we have made a study of the alveolar changes found in human lungs under various pathologic conditions and the alterations in animal lungs resulting from spontaneous diseases.

The Normal Pulmonary Alveolus in Man and Animals

Human embryologic studies suggest that the epithelial lining of the terminal vesicles begins to disappear about the end of the fifth month of fetal life (Barnard and Day,[11] Palmer,[12] and others); prior to that time, the lung has a distinct tubular or glandular appearance. Policard [13] pointed out that as late as the sixth month the lung is really a bronchiolar structure in a mesenchymal stroma; no true alveoli are present before that time and those that develop thereafter are not lined by epithelium. A different view was expressed by Bensley and Groff,[14] Zeldes,[15] and others, who believed that merely a transition from cuboidal to flattened epithelium occurs in the alveoli during fetal life.

Until respiration takes place, the extent and character of the alveoli

* Received for publication, January 18, 1943.

are poorly defined microscopically, because the potential air spaces are not expanded. In the lungs of newborn infants, who have died soon after birth from nonrespiratory causes, most of the alveolar walls are somewhat thick and cellular. They are composed of capillaries with a typical endothelial lining. Frequently a fairly characteristic cell, probably mesenchymal in origin and distinguishable with difficulty in the normal adult lung, is found in the alveolar wall and in the interstices between the capillaries. This cell resembles the endothelial cell in that its nucleus is pale, vesicular, fairly large, and usually oval; it differs from the endothelial cell in that it is less definitely related to a capillary and has an abundant clear or very slightly granular cytoplasm, which is irregular in contour. This is the so-called "septal cell," a term introduced into the American literature by Lang [16] in 1925. This term corresponds to the word "epicyte," introduced by Clara [17] and applied by Macklin,[18] and to the designation "alveolar cell," commonly used for tumors believed to arise from these cells, namely, "alveolar cell tumor" or "alveolar cell carcinoma." According to the opinion expressed by Maximow and Bloom,[8] Lang,[16] Fried,[19] Loosli,[20] and others, there is no epithelial lining in the alveoli during normal postnatal life. This is contradictory to the views of Miller,[10] Bremer,[21] Cooper,[22] and others, who believed that a continuous epithelial lining exists, and to those of Aschoff,[23] Bargmann,[24] Seemann,[25] and others, who thought that the isolated septal cells in the capillary niches are epithelial. Clara [17] and Macklin [18] stressed the origin of these cells from entodermal epithelium. These various divergent opinions were clearly summarized at a round table conference conducted by Macklin.[26]

The epithelium of the respiratory tract appears to terminate more or less abruptly at the beginning of the alveolar ducts. The walls of both the alveoli and ducts are composed of capillaries in a delicate reticular and elastic stroma. The septal cells scattered therein are usually distinguished easily from the bronchiolar epithelium.

The normal lung structure in animals, as observed chiefly in cattle, sheep and hogs, and to a less extent in dogs and cats, is similar to that in human beings—the alveoli and ducts consist of capillaries in a delicate reticulo-elastic stroma. Although isolated septal cells are present, no definite alveolar lining is evident in the lungs from healthy mature animals.

The Human Alveolar Lining under Pathologic Conditions

Introduction

The human material was selected from a series of 4,000 necropsies, which had been performed at the Colorado General Hospital. The

descriptions are based on paraffin-embedded lung sections, 6 μ thick, stained by the hematoxylin and eosin method. In certain instances, thicker sections, to a maximum of 25 μ, were stained for elastic and reticulum fibers, fat droplets, pigment, and glycogen. We are of the opinion that careful study of such routinely prepared lung tissue offers abundant information on this subject.

The changes to be described were observed in lungs with pneumonias, infectious granulomas, neoplasms, and miscellaneous conditions. Some idea of the frequency of these changes for each condition will be given, but no actual statistics can be furnished. The number of specimens of certain diseases was too small to be of value statistically. Our findings are based on sampling routine material rather than on exhaustive analysis of such disease. We feel that such sampling, even though incomplete, provides a fairly accurate impression as to the frequency of the changes. The terms "uncommon," "fairly common" and "frequent" will be used to denote the comparative frequencies. It is hoped that this attempt to correlate the fundamental facts and observations as to septal cell proliferation will stimulate further investigation.

Pneumonias

Bacterial. In textbooks on pathology (Boyd,[3] Karsner,[4] MacCallum,[5] Aschoff,[27] Lauche [28]) mononuclear phagocytic proliferation is described in the stage of resolution of lobar pneumonia. Some authors felt that these phagocytes are really desquamated epithelium, but placed no emphasis on distinct lining-formation in this disease. In our experience, septal cell proliferation and the formation of a definite lining are not observed in red or gray hepatization *per se*. They may occur in any stage, however, if the pneumonia is accompanied by other conditions. Figure 1 is an example of such proliferation in red hepatization, associated with chronic passive congestion of the lungs secondary to subacute bacterial endocarditis. Fibrin, erythrocytes, polymorphonuclear leukocytes, and mononuclear phagocytes are present in the lumina of the alveoli; the alveolar walls exhibit distinct but incomplete lining by low cuboidal cells. In the later stages of lobar pneumonia, especially when it is complicated by delayed resolution and organization, lining formation may readily occur. Figure 2 shows this feature in a chronic organizing pneumonia. In bronchopneumonia of bacterial origin, lining is uncommon during the early stages just as in the lobar form, but it may occur during delayed resolution and organization. Scattered septal cell swelling, however, may be observed in any stage (Fig. 3).

Viral. In pneumonia caused by viruses, proliferative characteristics

of the septal cells have been described repeatedly. MacCallum [29] reported such reactions in measles during World War I. In influenzal pneumonia desquamative and proliferative characteristics of the septal cells have been observed. However, in these pneumonias it is impossible to exclude the part played by complicating bacteria. Güthert [30] reported proliferative reactions in the septal cells in the pneumonia of psittacosis, as also noted recently by Appelbaum and Ackermann.[31] Feyrter [32] described septal cell proliferation in the pneumonia complicating pertussis; and Goodpasture, Auerbach, Swanson and Cotter,[33] though not stressing this feature, illustrated it in their photomicrographs of pneumonia in pertussis and measles. We [34] reported septal cell swelling, proliferation, and lining in pneumonia in chickenpox. Figures 4 and 5 demonstrate these features in pertussis and chickenpox, respectively. In summary, septal cell proliferation and alveolar lining are frequently observed in pneumonias associated with virus infections.

Chemical. Lipoid pneumonia, due to aspiration of oily substances, has received much attention in recent years (Goodwin,[35] Gowar and Gilmour,[36] Graef,[37] Ikeda [38]). The changes are of mixed acute and chronic character. Whereas Goodwin interpreted the cellular proliferation as arising from the "alveolar epithelium," Graef and Ikeda believed that it resulted from downgrowth of bronchiolar epithelium; Gowar and Gilmour, on the other hand, traced it to fixed histiocytes of the alveolar walls. In Figure 6 we offer an example of lipoid pneumonia showing prominent cuboidal-cell linings in the alveoli. In this instance there was no evidence of bronchiolar participation. Delafield and Prudden [7] described and illustrated pulmonary changes following nitric oxide poisoning in which the alveoli displayed similar lining.

Aspiration Other Than Lipoid. Good examples of septal cell proliferation in nonlipoid aspiration were not observed. This may be related to the factor of time, since such patients rarely live long enough following the development of aspiration pneumonitis to produce septal cell growth.

War Gases. We have had no experience with this type of inflammation and were unable to find reports in connection with the current war. Groll [39] stated in this regard that the "alveolar epithelium" became enlarged and formed a prominent lining in the bronchopneumonia due to phosgene poisoning in World War I.

Physical Agents: X-Rays and Radium. Warren and Gates [40] studied the pulmonary response to these agents in experimental animals and correlated the changes with those observed in man. They examined four species, the dog, pig, rabbit and rat, and noted various stages

of irradiation reaction up to a degree fairly constantly seen in man. Rupture and reduplication of the elastic framework, a hyaline lining membrane, and epithelial anaplasia of both bronchogenic and alveolar origin were found. Similar observations were made by Bauer and Schraer.[41] We had one example in our series, that of a woman, 40 years of age, to whom irradiation therapy had been administered because of a bronchial polyp that had undergone malignant change. Figure 7 illustrates the septal cell proliferation and alveolar lining, mobilization of foamy macrophages in the alveolar lumina, edema, and interstitial fibrosis in this case.

Infectious Granulomas

Although septal cell proliferation occurs not infrequently in acute inflammatory disease, it is much more common in specific chronic inflammations. The changes have been observed with almost all types of granulomas, but we shall restrict ourselves to a description of them as found in the more important granulomas.*

Tuberculosis. Active tuberculous foci commonly exhibit perifocal septal cell proliferation and alveolar lining formation. Several authors have described this (El Gazayerli,[1] Karsner,[4] Pagel and Henke,[43] and others). Illustrations are shown in Figures 8 and 9. Figure 8 displays lining formation in and around typical tuberculous granulation tissue; the alveoli are distorted; some of the lined spaces are probably alveolar ducts. Figure 9 demonstrates acute tuberculous exudation with many foamy macrophages in the lumina and distinct ribbonlike cuboidal cell lining.

It is well known that in and around apical scars cuboidal cells frequently line the open spaces. The question whether all such scars are of tuberculous origin is unsettled and we prefer not to discuss it here. Figure 10 shows a large area of apical fibrosis, in which centrally located spaces or distorted alveoli are lined by cuboidal cells, most of which appear to be firmly attached to the wall. Lining formation is apparent also along the margins of the scar.

Syphilis. Pulmonary involvement by this disease is extremely rare, at least in this country. We have found only one case showing pulmonary gummas. In that instance there was moderate perifocal septal cell proliferation and lining, essentially similar to the formation around tuberculous granulomas. Wohlwill[44] has recently compiled an extensive series of cases of pulmonary syphilis from material in Portugal.

* Interstitial pneumonia caused by toxoplasmosis was reported recently by Paige, Cowen and Wolf.[42] In their report septal cell proliferation was described. We have had no experience with this disease.

He described and illustrated alveolar lining within areas of luetic fibrosis. In pneumonia alba of congenital syphilis such lining, in addition to interstitial thickening, is generally observed.*

Neoplasms

Primary. The question whether true tumors may arise from alveolar lining cells is not settled at the present time. Many authors believe that all cancers of the lung are bronchogenic in origin. Others, on the basis of alveolar cell studies under various influences, have advanced the possibility "that conditions might arise which would make of this cell the nucleus of a primary cancer of the lung" (Macklin [18]). In a recent general review we [45] summarized the evidence in support of the existence of a specific tumor that arises from the septal cells, lines the alveolar walls, and is entirely independent of the bronchial system. The tumor may be found in a multinodular or in a diffusely infiltrating form. We preferred the designation "alveolar cell tumor," because this term leaves open the question of the nature of these alveolar cells; the term "carcinoma" would ascribe the origin to entodermal epithelium. Twenty-five instances of malignant and 4 of benign tumors of this type were compiled from the literature; in 15 additional cases, most of which were definitely malignant tumors, alveolar origin was possible if not probable. The case of Smith and Gault,[6] to which our attention has been called recently, should be added to the malignant group. Our own material included 5 additional examples of the latter form and we think that the alveolar cell tumor is not as rare as heretofore believed. The histologic features have been described at length in the review previously mentioned; suffice it to say here that the alveoli are lined by cuboidal or columnar neoplastic cells with papillary protrusions. Figure 11 shows a neoplastic lining; some of the cells exhibit bizarre forms. Close relation to intra-alveolar cells of phagocytic character is apparent. The section is from a multinodular type of alveolar cell tumor in a male, 39 years old (reported by one of us [46] in 1941).

Proliferation of lining cells in inflammatory conditions to a degree suggesting benign neoplasm occurs rarely in man (Oberndorfer [47]) but much more frequently in animals. This will be discussed later.

Metastatic. Tumors involving the lungs from foci elsewhere are occasionally surrounded by a perifocal septal cell reaction. This is to be distinguished from the actual lining of alveoli by neoplastic cells, a feature observed now and then in neoplasms which metastasize to

* An excellent illustration is furnished in Figure 423, p. 745, of the 7th edition of MacCallum's textbook.[5]

the lungs (Stahr,[48] Klotz [49]) and in bronchogenic tumors, which utilize the alveolar walls for a framework.

Miscellaneous Conditions

Silicosis. Septal cell proliferation in the alveoli surrounding silicotic nodules is commonly found and has been described by other workers. The alveoli bordering small discrete foci are frequently distorted and stretched as a result of the contraction of the nodule. In such cases the lining may be incomplete and only the wall directly adjacent to the focus may show an epithelioid, ribbonlike covering. Massive conglomerate silicosis rarely exhibits intrafocal lined spaces; cuboidal cells, however, are apparent in the alveoli adjoining the larger scars. Our material comprised about 50 cases of this disease. An example is offered in Figure 12.

Vascular Disturbances. In chronic passive congestion of the lung, septal cell swelling is frequently observed. True lining formation is uncommon, although noted by us in a few instances. In Figure 13 the alveolar lumina are filled with pigmented macrophages and the walls are bordered by cuboidal cells.

The alveoli around pulmonary infarcts occasionally exhibit septal cell proliferation and lining formation, particularly after supervening inflammation and fibrosis. Fischer [50] described this thoroughly in 1922. If the infarct is acute and death occurs rapidly, septal cell proliferation may not be found.

Scars of Undetermined Origin. The presence of a cellular lining in and around pulmonary scars is fairly common and is similar to that observed in tuberculosis and silicosis. Within larger scars one cannot be certain whether the lined spaces are really distorted alveoli, terminal bronchioles, or tissue clefts. The latter possibility is an interesting one. We have been unable to find a discussion of this question in the literature. The tendency of mesenchymal cells to form a continuous lining of open spaces is well demonstrated in certain tumors, notably synoviomas (Black [51]).

Atelectasis. In acute pulmonary collapse, septal cell swelling may be marked, but true lining formation is usually absent. Chronic atelectasis and fibrosis are often accompanied by septal cell proliferation and lining formation.

Pulmonary Inflammation in the Newborn. The alveolar walls in the newborn appear more cellular and thicker than in later life, as previously mentioned. The lumina are relatively smaller and the septal cells are more prominent. However, a distinct and complete alveolar lining does not form commonly under the influence of acute inflammation.

Pleurisy and Empyema. A definitely increased tendency toward proliferation of the septal cells of alveoli bordering the visceral pleura has long been recognized. This occurs under various conditions. No satisfactory explanation has been advanced and there is no evidence of penetration by the surface mesothelium. In pleural inflammatory diseases lining is frequently seen in the subjacent alveoli (Fig. 14).

Lung Abscess and Gangrene. Perifocal septal cell proliferation and lining are not infrequent in association with abscess and gangrene of the lung, especially in the more chronic forms.

Pulmonary Alveolar Lining in Animals under Pathologic Conditions

Acute Inflammation

In the older literature Bosc [52] and Borrel [53] pointed out the existence of "alveolar epithelial" proliferation in sheep pox; more recently Thorp and Hallman [54] reported a similar reaction in pneumonia in calves. We have examined 25 specimens of spontaneous acute pneumonia in different species, such as the hog, lamb, fox, goat, cat, horse and cow. An unusual opportunity was afforded by an instance of pneumonia in a tiger. Several cases of pneumonia in turkeys also were examined. The etiologic agents varied. In the early phases of pneumonia, septal cell swelling was commonly observed. Actual lining, however, could be seen in only a few instances of acute pneumonia.

In experimental work in animals various agents have been used successfully to produce acute inflammation with proliferative reactions of the septal cells. Among the etiologic factors, bacteria, viruses, toxins and chemicals may be mentioned. The exudate in such experiments frequently shows a predominance of mononuclear cells. We discussed this subject to some extent in a previous report [34] and, for comprehensive details, refer the interested reader to the paper by Ross.[55]

Chronic Inflammation

The total material consisted of 26 cases. One of us (C. L. D.) has observed at least 1,000 examples of chronic pneumonia in sheep on the slaughter floor. Together we have studied 20 such cases grossly and microscopically. The other chronic infections in our series were in the cow, goat and hog.

Chronic Progressive Pneumonia in Sheep. This is the condition that has been variously designated as "jagziekte," "Montana chronic progressive pneumonia" and "Icelandic adenomatosis." Excellent descriptions have been given by Bonne,[56] Cowdry and Marsh [57] and Dungal.[58]

The disease is progressive over many months, sometimes over a year or more. Since many pathologists are unfamiliar with the condition, we shall describe the changes briefly. The lungs are heavy and on section display many solid, gray, tumorlike foci, which vary in size and shape and may involve almost the entire organ. The intervening tissue sometimes exhibits bronchopneumonia. Histologically, the picture is striking in its similarity to adenoma or even carcinoma. Cowdry and Marsh, and Dungal stated that several pathologists to whom they submitted sections made a diagnosis of carcinoma. In the tumorlike foci both alveolar and bronchogenic cellular proliferation occurs. Figure 15 shows alveoli and alveolar ducts, lined by cuboidal cells, which form occasional papillary projections into the lumina. The alveolar septa are thickened and infiltrated by chronic inflammatory cells.

Chronic Pneumonias and Granulomas in Other Species. Chronic pneumonia continuing over many months, as observed in sheep, is infrequently seen in other species. In our collection we have examined 6 cases—3 in cattle, 2 in goats and 1 in a hog. Theiler [59] described a related condition in horses. Olafson and Monlux [60] recently reported similar features in toxoplasmic pulmonary infection in a cat, and Gruber [61] observed them in verminous pneumonia in deer. In such instances the alveolar septal cell and bronchogenic proliferation closely simulate the histologic changes described in the chronic progressive pneumonia in sheep. We have noted perifocal septal cell proliferation also in animals of various species that had suffered from such rare chronic infections as actinobacillosis, actinomycosis, aspergillosis, coccidioidal granuloma, distomiasis and verminous pneumonia. Intrafocal lining is seen sometimes in the more fibrosed areas of these inflammatory conditions but far less frequently than in the chronic progressive pneumonia of sheep.

Neoplasms

Although we have personally observed several cases of alveolar cell tumor in man and have looked for its counterpart in animals, particularly in sheep and cattle, we have been unable to find satisfactory examples. The septal cell proliferations in the chronic progressive pneumonia of sheep are not considered true neoplasms by most authorities in this field. To us this interpretation seems correct. In other species, however, namely, mice, tumors that arise from the alveolar walls either spontaneously or experimentally have been described (Wells, Slye and Holmes,[62] McDonald and Woodhouse [63]). Grady and Stewart [64] experimentally produced tumors in strain A mice by subcutaneous injections of methylcholanthrene and of dibenzanthracene.

The tumors were of multicentric origin and the histogenesis was described as follows in the abstract which they submitted:[65]

"Immediately preceding the development of tumors and accompanying the early stages of their formation was a notable proliferation of large mononuclear cells from the alveolar walls. . . . . Frequently they would form columns of various length partly or completely lining the alveolar space, or they might coalesce to form small groups. These groups might project into the alveolar lumen or occur within or on the septal wall. The adenomatous nodules appeared to develop through a combination of these processes. . . . . [they were] composed of more or less closely packed columns of cuboidal or low columnar cells with relatively large nuclei. . . . . few mitotic figures, ... cytoplasm . . . . . occasionally contained small particles of phagocytosed material."

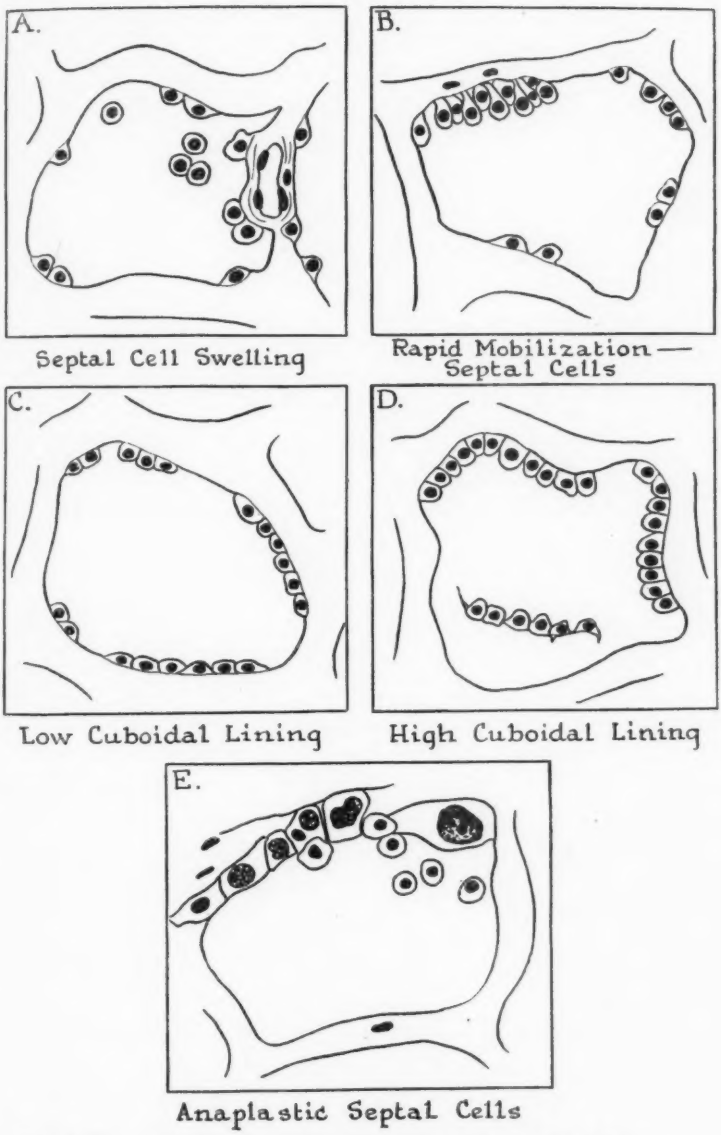
The authors hesitated to call these tumors "epithelial" because, after several transplants, they began to resemble spindle cell sarcoma. Wells, Slye and Holmes[62] also observed sarcomatous tendencies in metastases of spontaneous lung tumors in mice. Andervont[66] and Breedis, Robertson, Osenkop and Furth[67] noted, in serial transplants, transformation of such tumors from an epithelial to a spindle cell sarcoma-like growth. The latter authors pointed out that the alveolar lining cells of mice "have potentialities of assuming both epithelial-like and sarcoma-like forms."

Discussion

Developmental Stages of Lining. The alveolar cells accumulate readily under various pathologic conditions, as has been described in the preceding sections. All stages from simple swelling of the septal cells to the formation of a continuous epithelioid lining and to actual neoplastic growth (alveolar cell tumor) may be observed. We have illustrated the various stages diagrammatically in Text-Figure 1.

In early stages the nuclei of the septal cells become swollen and more prominent. They may be dark and homogeneous or pale, with a few chromatin granules and a dark nuclear membrane. The cytoplasm becomes more prominent, granular, or vacuolated and bulges into the alveolar lumen, although the cell remains continuous with the underlying ground substance (Text-Fig. 1, A). Such swelling can take place within a few hours, and has been verified in cases of massive acute postoperative atelectasis. Sometimes the septal cells appear to have enlarged and multiplied to a remarkable degree within a very short time. In such instances they may project into the lumen in clusters and their cytoplasm may become pear-shaped (Text-Fig. 1, B).

The next stage is actual lining. As the cells increase and their cytoplasm comes in contact with that of adjacent cells, they become low-cuboidal and form an indented, ribbonlike lining. At this point they assume an epithelioid appearance but usually remain attached to the



Text-Figure 1. Diagram of representative stages in septal cell activity.

underlying mesenchymal tissue. Special stains reveal no basement membrane in this or any other stage. The lining is apt to be incomplete, covering only a part of the alveolus (Text-Fig. 1, C).

Later the cells may become high-cuboidal with their attachment

to the underlying wall more delicate; the cells exfoliate occasionally in small numbers or in rows (Text-Fig. 1, D). One obtains the impression that to this stage the lining is not permanent and that it will disappear if recovery takes place.

Whenever the stimulating factor operates over a longer period of time, as in chronic inflammation, the proliferation is more apt to result in a complete, firmly attached lining, composed of higher cuboidal cells, which sometimes form papillary projections into the lumina. This lining appears more permanent, and such an assumption is supported by the fact that lined alveoli are found in old scarred areas.

In Text-Figure 1, E, anaplastic septal cells are shown with their attachment to the alveolar walls, as seen in an alveolar cell tumor (Fig. 11).

Although we have attempted to separate septal cell reactions into successive stages, we wish to emphasize that overlapping and combinations of these stages are commonly encountered.

Relation of the Mononuclear Phagocyte to the Alveolar Cell. There are several opinions as to the origin of the "dust cell" or alveolar phagocyte. According to one view, sponsored by Maximow and Bloom,[8] Lang,[16] Fried,[19] Clements,[68] and others, the septal cells provide the majority of these phagocytes. Other opinions differ and assign the origin to either the cells of the blood stream or the capillary endothelium. Our material suggests that a high percentage of the mononuclear phagocytes arises from the septal cells. This study has not answered the question why the septal cells tend predominantly, under some conditions, to form alveolar linings; under others, to develop mainly into free phagocytes; and, in yet others, to manifest a combination of both features. It would appear that in certain acute inflammatory diseases or in the early stage of chronic inflammation, phagocytic properties are more pronounced. In these conditions, however, septal cell lining may be developed. The lining cells can become detached to form free phagocytes or they may remain attached to the wall and yet be phagocytic, as illustrated by occasional fat droplets and iron and dust particles in their cytoplasm. In the attached cells, these phagocytic properties are limited and are much less evident than in the free forms. Figure 16 is an example of swollen septal cells in a sheep; the cells are enlarged and are in the process of detachment from the wall to become free phagocytes. In old inactive conditions, particularly in pulmonary scars, the lining cells are inert and appear to have lost the power of phagocytosis and of producing phagocytes.

Relation of Septal Cell Proliferation to Specific Lung Regions. Lining formation is more readily found in certain locations than in others.

The septal cells in the alveoli subjacent to the visceral pleura show a pronounced tendency to proliferation, as mentioned earlier. Furthermore, the tendency is marked among the cells along the interlobular septa and in perivascular and peribronchial areas, but to a lesser degree. Macklin [18] has demonstrated experimentally that the septal cells are more concentrated in the alveolar walls adjacent to these regions. This is of interest in its relation to the findings of Wells, Slye and Holmes,[62] who observed in mice that spontaneous and experimentally produced lung tumors arose almost invariably in locations near the pleura.

Differentiation between Septal Cell and Bronchial Epithelial Lining. This distinction is important. We have observed both forms in man and in animals, and in most instances were able to differentiate between them without the aid of serial sections. Bronchiolar epithelial downgrowth into the alveolar ducts or alveoli may be of two types, squamous and columnar. The squamous type is easily distinguished histologically and is present in the form of irregular cell nests; this type of epithelium is metaplastic. In a few blocks which we studied serially, an early unsuspected squamous cell carcinoma was discovered. Columnar epithelial downgrowth is more important in its relation to our subject, because this type of epithelium may resemble the lining septal cell. We noted the differentiation best in the lungs of sheep that had suffered from chronic progressive pneumonia; this disease offered an opportunity to study both types of cells side by side in the same section. Bronchogenic epithelial downgrowth could be readily identified in most cases, because of the close relation to a central bronchus or bronchiole. The cells lining such spaces were high-columnar and were similar to bronchogenic epithelium, that is, the nuclei were oval or rod-like and not infrequently were arranged in layers; usually the cytoplasm was not clearly demarcated. Furthermore, the lumina of spaces lined by bronchogenic epithelium did not contain the mononuclear phagocytes to any such degree as that observed in zones of septal cell proliferation; the transition between the lining cells and mononuclear phagocytes was absent. Proliferating bronchiolar epithelium often covered spaces in a manner which produced thick collars of cells without evident cellular papillary projections. In summary, although the alveoli may become lined not only by autochthonous cells but also by bronchogenic epithelium, each type manifests fairly distinct characteristics.

Relation to Debatable Questions in Normal Histology. It is not our purpose to furnish conclusive evidence concerning debatable topics in normal pulmonary histology, since this study is based on pathologic material. Our findings, however, appear to support the view of Maxi-

mow and Bloom,[8] Lang,[16] Fried,[19] Loosli,[20] and others, with respect to the structure of the normal alveolar wall, namely, that no true continuous epithelial lining exists in adult life. The capillaries appear to be contained in a ground substance in which occasional septal cells are present. The question whether these cells are of mesenchymal origin or are remnants of the fetal entodermal epithelium cannot be decided on the basis of our studies, although the first assumption appears to be preferable.

Histogenesis of the Alveolar Lining. Histologic findings indicate that the proliferative process leading to the production of lining takes place within the mesenchymal tissue of the alveolar wall. Mitotic figures, as found under experimental conditions (Macklin [69]), are almost never observed. The lining cells become visible within the ground substance and proceed to cover the surface of the alveolar wall: thus a mesenchymal rather than an entodermal lining is formed. The genesis of cellular multiplication is not at all clear; proliferation of pre-existing septal cells certainly accounts for most of it, but participation of hematogenous cells cannot be excluded. At any rate, the potentialities of this mesenchymal tissue appear great, and large numbers of cells, presumably not present before, or at least not previously discernible, may become visible rapidly in the mesenchymal ground substance. The cellular mobilization is so striking that one is tempted to resort to Grawitz' [70] theory of "slumbering cells" in the connective tissue.

Factors Stimulating the Formation of Lining. In small, early, bronchopneumonic foci one may observe variable, but more or less marked, transient septal cell swelling. In certain other forms of acute inflammation, notably those due to virus infections, the irritant factor is capable of producing septal cell proliferation and actual lining. The stimulus appears to be some change in circulatory conditions as a result of infection. Ricker [71] demonstrated that in prolonged disturbances of circulation characterized by marked slowing of the blood stream, called by him "peristatischer Zustand," stimulation of the mesenchymal tissue elements occurs. This may be the explanation, at least in part, for the septal cell stimulation and alveolar lining formation, particularly in chronic diseases. In alveolar cell tumors the causative force is obscure.

Interference with internal respiration appears to be an important factor in the formation of alveolar lining as mentioned by Aschoff.[23] He stated that alveoli that are collapsed or filled with exudate show marked tendency toward the proliferation of what he called "alveolar epithelium." The physicochemical aspects of this subject were considered by Young.[72]

Functional Disturbances in Lined Alveoli. Lined alveoli are probably unable to function properly. Zeldes [15] emphasized this on the basis of a study of the lungs of newborn infants, and believed that an extensive cuboidal lining was incompatible with respiratory function and led to the death of the infant soon after delivery. We have pointed out that in acute inflammatory conditions the lining probably disappears with recovery. The inert lining in the chronic inflammatory conditions described earlier may interfere with respiratory function. In scars the lined spaces are probably cut off from the bronchial tree and are nonfunctional.

Summary

1. The pulmonary alveoli in man and in animals were studied with special regard to the character of the lining.

2. No evidence of continuous "alveolar epithelium" was observed in normal adult lungs; only occasional scattered septal cells were seen.

3. Epithelium-like lining cells were found under various spontaneous pathologic conditions.

4. The origin and development of the epithelium-like alveolar lining cells were discussed; their probable mesenchymal character was indicated.

We wish to express our appreciation to Drs. C. C. Macklin and H. G. Grady for many valuable suggestions.

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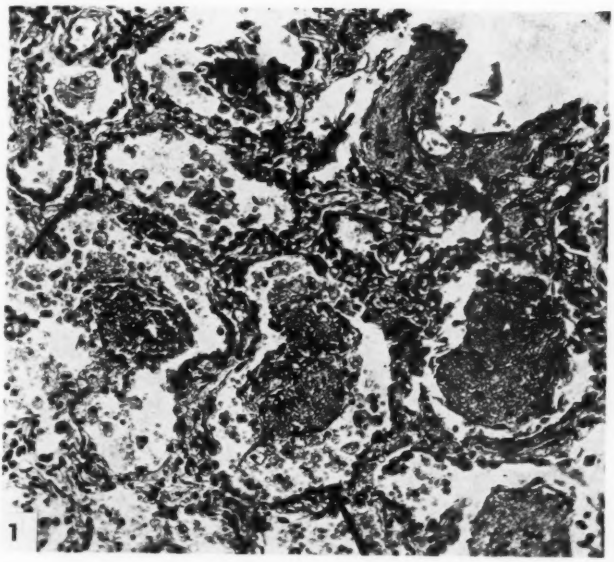
DESCRIPTION OF PLATES

PLATE III

FIG. 1. Lobar pneumonia, stage of red hepatization, complicated by chronic passive congestion. Alveoli are lined by low cuboidal cells and filled with exudate. × 140.

FIG. 2. Chronic pneumonia. Alveolar walls are thickened and lined by cuboidal cells. × 280.

FIG. 3. Bronchopneumonia. Alveolar walls exhibit many swollen, prominent septal cells; lumina contain exudate with polymorphonuclear leukocytes, fibrin and occasional foamy phagocytes. × 175.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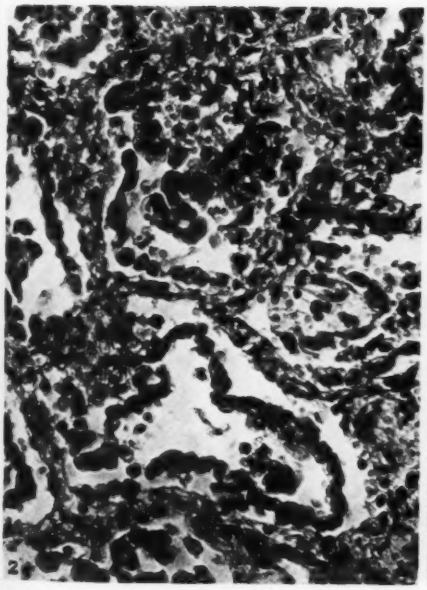
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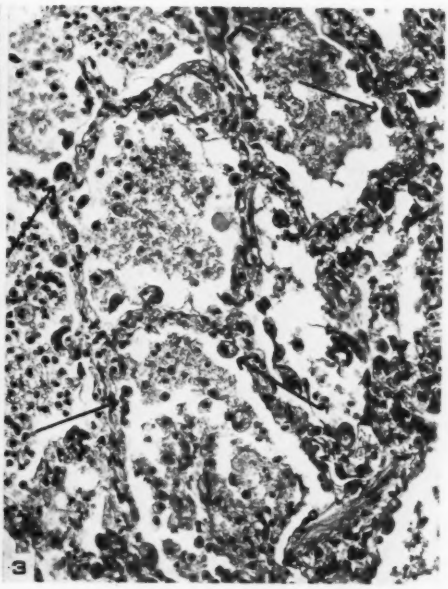
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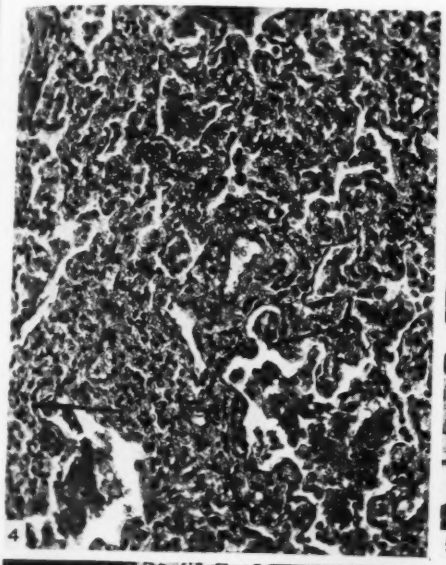
PLATE 112

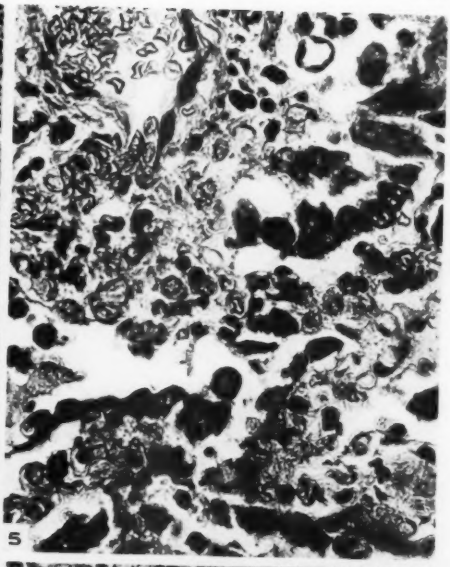
FIG. 4. Pneumonia complicating pertussis. Alveolar walls are lined by low, sometimes flattened, septal cells; many air sacs are atelectatic. Mononuclear cells predominate in the exudate. × 140.

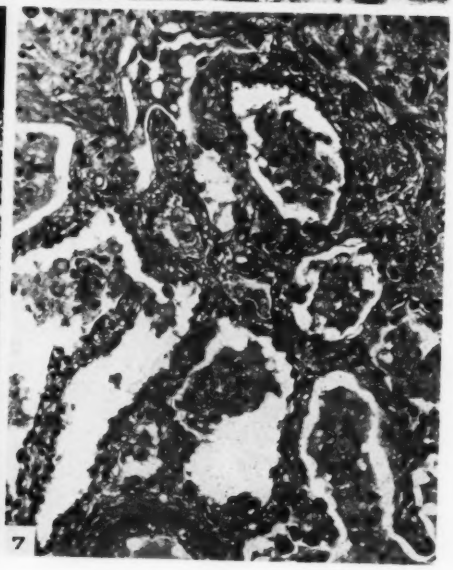
FIG. 5. Chickenpox pneumonia. Alveolar walls show partly exfoliated lining. × 350.

FIG. 6. Lipoid pneumonia. Cuboidal lining is apparent in most alveoli. Lumina contain much free and phagocytosed fat. × 100.

FIG. 7. Pulmonary changes secondary to x-ray and radium treatment for malignant bronchial polyp. Alveolar walls are thickened and lined by low cuboidal cells. Foamy phagocytes are present in the lumina. × 155.







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The Pulmonary Alveolar Lining

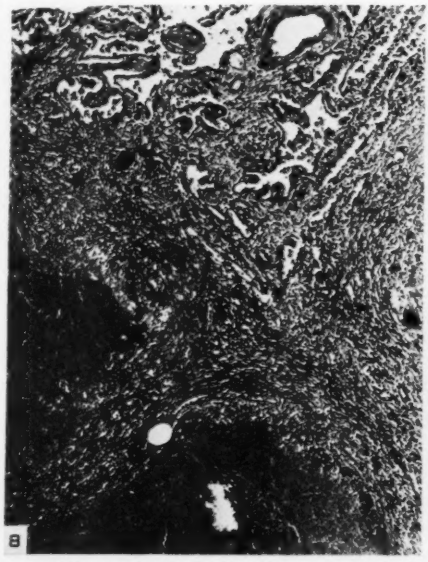
PLATE 113

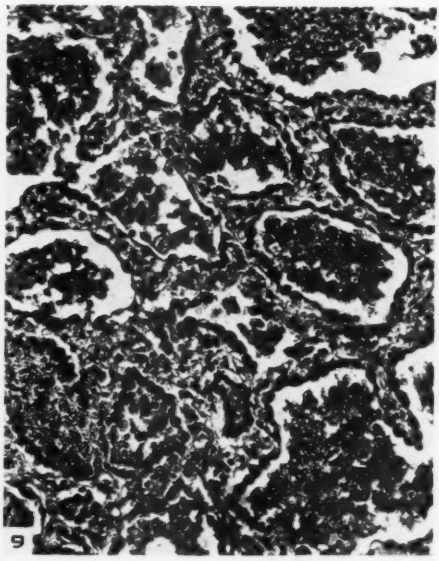
FIG. 8. Pulmonary tuberculosis. Marginal tuberculous granulation tissue is apparent around foci of necrosis. The adjacent alveoli are irregular and lined by continuous cuboidal cells. × 45.

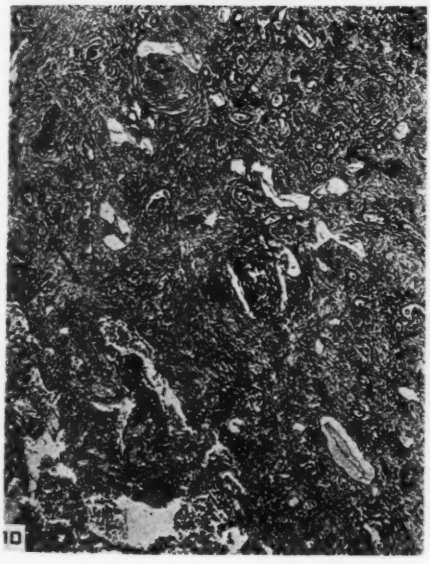
FIG. 9. Pulmonary tuberculosis, area of acute exudation. Alveolar walls are congested, thickened and lined by continuous low and tall cuboidal septal cells. Phagocytes, round cells and occasional polymorphonuclear leukocytes are present in the exudate. × 160.

FIG. 10. Apical scar. Distorted or constricted alveoli are present in a fibrous background. Cuboidal lining is displayed in the majority of such alveoli. × 45.

FIG. 11. Alveolar cell tumor. Alveolus shows anaplastic lining with close relation to foamy phagocytes. × 360.







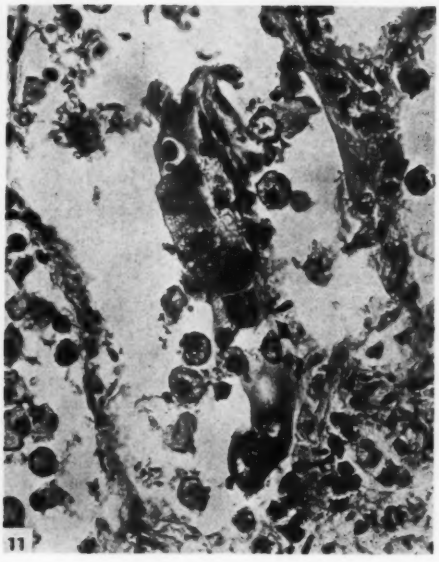


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PLATE 114

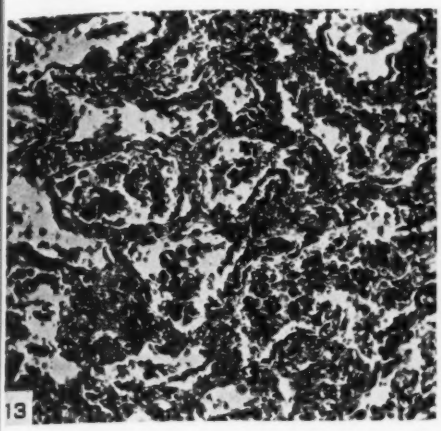
FIG. 12. Silicotic nodule. Marginal alveoli are emphysematous and exhibit ribbon-like cuboidal lining on the surface adjoining the nodule. × 115.

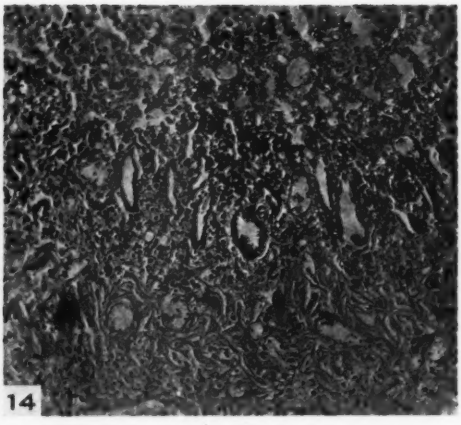
FIG. 13. Chronic passive congestion. Alveolar walls are covered by cuboidal cells and the lumina filled with "heart lesion" phagocytes. × 95.

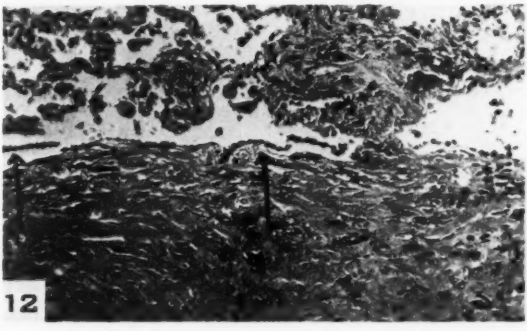
FIG. 14. Pleurisy. Visceral pleura is thickened and fibrous and subjacent alveoli are lined by cuboidal or columnar cells. × 110.

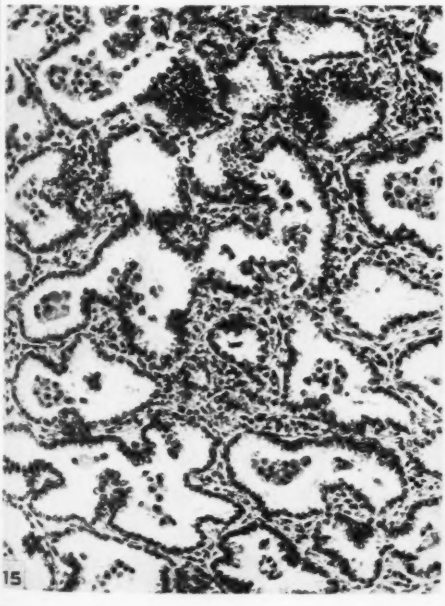
FIG. 15. Chronic progressive pneumonia in a sheep. Alveoli and alveolar ducts are lined by cuboidal or low columnar cells forming papillary projections into the lumina. Alveolar walls are thickened and show round cell infiltration. × 175.

FIG. 16. Acute pneumonia in a sheep. Alveoli have pear-shaped septal cells, projecting into the lumen in clusters. Polymorphonuclear leukocytes and foamy phagocytes are also present. × 410.









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EXPERIMENTAL BRAIN TUMORS

II. Tumors Produced with Benzpyrene*

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In experiments with pure crystalline 20-methylcholanthrene implanted in pellet form in the brains of C_3H male mice, a variety of gliomas and intracranial sarcomas were produced which have already been reported.[1] More recently another hydrocarbon, benzpyrene, was utilized for intracerebral implantation in both male and female mice of the C_3H strain, and with this chemical carcinogen also intracranial neoplasms were produced. These experiments were previously reported in preliminary form [2] and now are the basis of this detailed communication.

Other investigators have employed benzpyrene in attempts to produce brain tumors with invariably negative results in regard to gliomas. In 1936, Oberling, Guérin and Guérin [3] applied this hydrocarbon in crystalline form to the brains of 10 adult rats, of which 3 animals developed pituitary adenomas after 10 months of survival. Another rat which received 1 drop of a 1:1000 oily solution of benzpyrene intracerebrally developed an atypical epitheliomatous tumor in the anterior lobe of the hypophysis. The authors maintained at that time that the tumors were produced by the chemical carcinogen because of the rarity of spontaneous pituitary tumors in rats. Three years later, however, Oberling, Sannié, Guérin and Guérin [4] conducted more extensive experiments with an oily solution of benzpyrene in guinea-pigs, rabbits, hens and rats in an effort to duplicate these results. They also implanted crystals of this chemical in the brains of 40 rats. The negative results forced them to confess that pituitary adenomas could not be induced by these methods and that the previously reported adenomas were spontaneous. Although the experiments were continued for over a year, no gliomas and only 12 intracranial and extracranial sarcomas developed.

In 1937, Askanazy [5] reported finding a piece of cartilage in the ventricle of a rabbit injected intracerebrally with crushed rabbit fetal tissue and with benzpyrene in olive oil. This animal lived only 22 days but another, treated similarly, which survived 8 weeks, was said to have had a chondroma in the lateral ventricle. One rabbit received an intracerebral injection of benzpyrene in beef fat and died 8 months

* Aided by a grant from The Jane Coffin Childs Memorial Fund for Medical Research.
Received for publication, January 15, 1943.

and 5 days later with a "chondrosarcoma" in the cerebellum. Considerable doubt may be expressed as to the neoplastic nature of any of these cartilaginous masses, for at least in the first two instances the cartilage probably represented part of the crushed fetal tissue.

Bertrand and Gruner [6] suspended benzpyrene in lanolin, paraffin oil and vaseline, injecting this material in doses of 5 to 15 mg. intracerebrally in rabbits. Although in certain instances a giant nuclear glial response appeared at the sites of injection, true gliogenous neoplasia was not produced. In a personal communication to Seligman and Shear,[7] Zylberszac stated that he failed to obtain tumors in 35 rats in which he introduced crystalline benzpyrene subdurally. In a similar communication,[7] Scherer revealed that he injected 0.1 cc. of a 1 per cent lard solution of benzpyrene into the frontal lobes of 70 white rats and gave 50 of the animals more than one injection. Granulomas were found in all cases but no neoplasms were seen, and lard itself caused the same reaction.

In view of the success that attended the experimental production of brain tumors with pellets of pure methylcholanthrene in C_3H mice,[1] the essentially negative results of all of the experiments with benzpyrene were surprising. But it must be noted that no one had used the mouse as the experimental animal and, further, that usually the carcinogen was diluted or contaminated with oily vehicles that set up foreign body reactions. Even when crystals of benzpyrene were employed they were not compressed into pellets for intracerebral implantation. In the following experiments, which took account of these factors, namely, the animal species and the pellet form of the carcinogen, brain tumors of a large variety were produced.

Experimental Method

A total of 51 mice of the C_3H strain, representing both sexes in about equal numbers, was employed in these experiments. The animals were between 3 and 4 months of age. Their care and diet were in every way similar to those of the earlier experiments with methylcholanthrene.[1] Likewise, the anesthesia, operative procedure and aftercare were the same, with the exception that the chemical carcinogen was implanted in the right cerebral hemisphere in each instance. As in the earlier experiments, the tumors of doubtful diagnosis which developed were transplanted to the subcutaneous tissues of other mice of the same strain. The help that was derived from these transplants in classifying the tumors has been utilized in the presentation of the data which follow.

The necropsy and histologic technics were similar in these and in

the experiments with methylcholanthrene. The carcinogen was 3,4-benzpyrene, obtained from Meurice, Union chimique belge, Brussels, Belgium. Without further purification, the canary yellow crystals were heated gently in a small beaker until they fused. When cooled, cubes of about 1 mm. diameter were cut with a knife and were employed, without further treatment, for implantation in the mouse brains.

Results

Of the 51 mice at the start of the experiments, 47 survived into the tumor-bearing age. The tumor incidence and classification are presented in Table 1.

Table I

Tumor Incidence and Classification

Total number of animals		47
Negative for tumor		19
Total number of tumors		28
Gliomas		14
Astrocytoma	1	
Ependymoblastoma	4	
Glioblastoma multiforme	3	
Medulloblastoma	1	
Oligodendroglioma	1	
Unclassified glioma	4	
Sarcomas		13
Extracranial fibrosarcoma	2	
Intracranial fibrosarcoma	9	
Rhabdomyosarcoma	2	
Mixed glioma and sarcoma		1

These experiments were completed after 458 days upon the death of the last surviving mouse. The average survival time for the animals which developed gliomas was 276 days as compared to 245 days for those which developed sarcomas. The related data are presented in Table II.

Many of the tumors produced with benzpyrene were similar to those which resulted from the intracranial implantation of pellets of methylcholanthrene. Since the latter were described and illustrated extensively in the earlier paper,[1] the same types of neoplasms produced with benzpyrene will be dealt with only briefly at this time. More attention will be given those tumors, like the ependymoblastomas, which have not been described previously.

Gliomas

Astrocytoma. Mouse 21. A gray, opaque, poorly defined tumor was present at the site of the benzpyrene pellet in the right cerebral hemisphere. The cells formed no definite pattern, being arranged in helter-skelter fashion. Each consisted of a small, deeply stained round

nucleus and inconspicuous cytoplasm with multipolar processes. There were few cells in mitotic division. Minute portions (1 mm. in diameter) of this tumor were transplanted into the subcutaneous tissues of the right flanks of 4 C_3H mice, but only one transplant grew after 3 months. The remaining three failed to grow even after 4 more months. Subtransplants were made of the one positive transplant, yielding two

TABLE II

Survival Time of Animals with Various Tumors

Gliomas, 14 animals: average survival time, 276 days

Mouse no.	Days' survival	Tumor type
10	359	Unclassified
12	286	Unclassified
17	222	Glioblastoma multiforme
19	384	Ependymoblastoma
21	238	Astrocytoma
22	230	Oligodendroglioma
26	329	Glioblastoma multiforme
35	189	Glioblastoma multiforme
37	272	Unclassified
38	230	Ependymoblastoma
40	319	Ependymoblastoma
43	174	Ependymoblastoma
50	452	Unclassified
52	183	Medulloblastoma

Sarcomas, 13 animals: average survival time, 245 days

Mouse no.	Days' survival	Tumor type
3	208	Intracranial fibrosarcoma
5	267	Intracranial fibrosarcoma
6	222	Rhabdomyosarcoma
8	153	Intracranial fibrosarcoma
13	239	Rhabdomyosarcoma
14	153	Extracranial fibrosarcoma
16	458	Intracranial fibrosarcoma
18	458	Intracranial fibrosarcoma
20	171	Intracranial fibrosarcoma
30	170	Intracranial fibrosarcoma
45	275	Extracranial fibrosarcoma
47	147	Intracranial fibrosarcoma
51	267	Intracranial fibrosarcoma

Mixed glioma and sarcoma

Mouse no.	Days' survival	Tumor type
1	208	Mixed glioma and sarcoma

positive "takes" out of 4 animals. The cells in the tumor transplants were polygonal and had many polar processes. They were often arranged around irregular spaces which were filled with pale pink (in hematoxylin and eosin preparations), colloid-like material.

Ependymoblastoma. Mouse 19. At necropsy the brain of this animal had a small tumor nodule in the right lateral ventricle attached to the lateral wall and infiltrating the right cerebral hemisphere. In places the ventricular ependyma was replaced by broad bands of tumor

cells which were carrot-shaped and invaded the choroid plexus. The cells which infiltrated the hemisphere were frequently arranged as collars around blood vessels and formed rosettes whose centers consisted of granular débris. Numerous cells were in mitotic division.

Mouse 38. Two days before this animal died it became inactive, appeared to be sick and developed a deformity of the head. At necropsy the cranial sutures were found separated and the occipital bone thin and bulging. A roughly spherical tumor mass was found in the right lateral ventricle with the pellet of benzpyrene (B.P.) still present in the upper part of the tumor (Fig. 1, B.P. 38). Microscopically the ventricular ependyma was found to have proliferated in several places. The cells were typically carrot-shaped and were often arranged around blood vessels.

Subcutaneous transplants of this neoplasm in 4 C_3H mice yielded three positive "takes" in less than a month. Subtransplantation was carried out twice with all of the animals showing tumor growth in less than 30 days. The tumor transplants were even more characteristic of ependymoblastoma than the original neoplasm. All of the cells were carrot-shaped and formed typical rosettes. Many cells were in mitotic division.

Mouse 40. The head of this animal became asymmetrical 2 days before it died, when the cranial sutures were found widely separated and the right half of the cranium was soft. The tumor was situated in the right cerebral hemisphere, of which the ventricle was dilated and filled with an excess of clear fluid. The choroid plexus as well as the leptomeninges were infiltrated with tumor cells. Pear-shaped cells radiated from blood vessels and formed rosettes with centers that were fibrillary, acellular and pink-staining. Even in the meninges the tumor cells formed typical rosettes (Fig. 2). Mitoses were present in abundance. Subcutaneous transplants of this neoplasm gave rise to growths which were typical of ependymoblastoma (Fig. 3).

Mouse 43. Four days before this animal died it began to run in circles and to fall to the right. Its head was deformed due to separation of the cranial sutures. A tumor replaced the posterior half of the right cerebral hemisphere and resulted in a generalized dilatation of the ventricular system. The tumor cells were carrot-shaped and were arranged in rosette formations with many cells in mitotic division. This tumor was kept alive through 4 generations of transplants involving a total of 16 animals, when further transplantation was arbitrarily stopped. Every transplant grew, reaching a minimal diameter of 1 cm. in approximately 1 month's time. The characteristic cellular pattern was maintained through all generations of transplants.

Glioblastoma Multiforme. Mouse 17. A small palpable nodule became evident beneath the scalp in the right parietal region of this animal 10 days before death. This tumor grew rapidly and at necropsy was found to arise from the right cerebral hemisphere, projecting beneath the scalp through an erosion of parietal bone (Fig. 1, B.P. 17). Microscopically the tumor was diffusely infiltrating glioma with evidence of invasion of the meninges as well as of the ventricular system. The cells were pleomorphic and there were many multinucleated giant cells as well as cells in mitotic division. A characteristic arrangement of the cells was in pseudopalisades around zones of necrosis.

Three generations of subcutaneous transplants of this tumor were kept alive, the first transplants showing evidence of growth in about 6 weeks and the latter two, in a little over 3 weeks. The transplanted tumors failed to develop giant cells and pseudopalisades. The structural pattern assumed by the cells resembled that of a piloid astrocytoma.

Mouse 26. This animal died without revealing any external evidence of an intracranial neoplasm. Such a tumor, however, was found at necropsy surrounding the pellet of benzpyrene in the subcortex of the right cerebral hemisphere. Microscopically this neoplasm was a typical glioblastoma multiforme, with multinucleated giant cells, spongioblasts and necrotic foci surrounded by cells of pseudopalisade formation. Small round cells formed collars around blood vessels in the tumor.

Mouse 35. At death there was no deformity of the head of this animal. The skull was intact, but there was a slight separation of the cranial sutures. A gray, opaque tumor containing minute hemorrhages was present in the right parieto-occipital region of the brain (Fig. 1, B.P. 35). Neoplastic cells were found invading the choroid plexus microscopically. There was a great diversity of cell form and size, with many elements in mitotic division. Occasional multinucleated giant cells were present. Spindle-shaped spongioblasts formed pseudopalisades around foci of necrosis.

Medulloblastoma. Mouse 52. There was nothing on external examination of the skull of this animal to indicate the tumor which was situated in the parieto-occipital region (Fig. 1, B.P. 52). When sectioned, the central portion of the neoplasm was found to be hemorrhagic. There was no neoplastic involvement of the cerebellum. The cells had a vague resemblance to lymphocytes: the nuclei were round and deeply stained in inconspicuous cytoplasmic bodies. Mitotic figures were exceedingly numerous. The cellular infiltration receded so

gradually that there was no real demarcation between the neoplasm and the adjacent nervous tissue. The perivascular spaces of many cerebral vessels contained nests of tumor cells. In the main body of the new growth as well as in its perivascular extensions, a tendency to form pseudorosettes was a conspicuous feature of the cells. In spite of the rather unorthodox location of this neoplasm, its cellular composition and structural pattern permit no diagnosis other than medulloblastoma.

Oligodendroglioma. Mouse 22. Nearly 2 weeks before this animal died, a small mass was visible on the right side of its head. This increased in size only slowly, and at necropsy was found to emanate from the burr hole in the skull. The benzpyrene pellet was found lying superficially in the cortex of the right occipital lobe and did not appear to be surrounded by tumor. On serial microscopic examination, however, the region of the pellet was occupied by typical oligodendroglia, with centrally situated round nuclei and colorless cytoplasm which produced the appearance of a perinuclear halo in each cell.

Within 2 months after subcutaneous transplantation of this tumor into 4 C_3H mice, all animals showed evidence of a positive "take." Subtransplants of the tumor from the flank of 1 animal into 4 others yielded three positives at the end of 2 months. Further subtransplantation yielded once more three out of four positive "takes" in a little less than 2 months. With minor variations the microscopic structure of all of the transplants resembled the original brain tumor. The variant which appeared in 2 animals consisted of bands of spindle-shaped mesodermal cells and reticulin in addition to the oligodendroglia.

Unclassified Glioma. Mouse 10. This animal died without displaying a change either in its behavior or the shape of its head. Necropsy, however, revealed a gray-brown tumor in the right cerebral hemisphere. The cells were nearly all spindle-shaped and had elongated nuclei. Frequently they were arranged in concentric rings or bands around blood vessels, resembling spongioblasts quite closely. Mitotic figures were moderately numerous. There was invasion of the choroid plexus. Preparations stained by the Wilder method failed to disclose connective tissue reticulin fibers. The unexpected death of this animal precluded further study of this neoplasm by the transplantation method.

Mouse 12. The unexpected death of this animal occurred without any cranial deformity or change in behavior. The intact skull having been removed, a gray-brown tumor was found in the posterior half of the right cerebral hemisphere. When sectioned, the central portion of the tumor was seen to be hemorrhagic. The histologic appearance of the

neoplasm varied in different parts. In some zones the cells were polygonal and had abundant pink cytoplasm and large, chromatin-rich nuclei. In other zones bizarre-shaped, multinucleated giant cells were conspicuous. In still others, mononuclear cells with foamy cytoplasm and cytoplasmic vacuoles predominated. These various cellular elements infiltrated the nervous tissue diffusely. Perivascular lymphocytic infiltration was noted in several places. Cells in mitotic division were seen rather frequently. In spite of certain resemblances to glioblastoma multiforme, this glioma is left unclassified. Transplants were not available to aid in diagnosis.

Mouse 37. This animal became inactive and appeared to be sick 1 week before it died. It held its head to the left, but there was no cranial deformity. Section of the brain disclosed a dilatation of the ventricular system, slightly more marked in the right lateral ventricle (Fig. 4). A gray, translucent tumor nodule was present at the site of the benzpyrene pellet which lay embedded in the septum pellucidum just beneath the corpus callosum. Serial microscopic preparations of the brain showed that the tumor nodule was composed mainly of large, round mononuclear glial cells (Fig. 5). Some cells were considerably smaller; a few had short, thick cytoplasmic processes; some were in mitotic division. Here and there a cell was multinucleated and of giant size. Scattered throughout the brain were vessels with small round cells in their perivascular spaces. Tumor cells had infiltrated the leptomeninges and lay in a cluster within the third ventricle (Fig. 7). Preparations of a portion of the tumor nodule stained by the Wilder method for mesodermal reticulin were entirely negative (Fig. 6).

Although certain features of this neoplasm were suggestive of glioblastoma multiforme, its general microscopic appearance did not warrant this classification. Moreover, the transplantation experiments gave little aid in this direction. In the first transplant generation, 3 out of 4 mice developed subcutaneous tumors, but only after 3 months. All 4 animals of the second transplant generation developed tumors within 5 weeks, but in no instance were the transplants more characteristic microscopically than the original brain tumor.

Mouse 50. There was no microscopic evidence of a neoplasm in the brain at necropsy. Microscopic serial sections of the entire cerebrum revealed a gliogenous tumor adjacent to the pellet space in the subcortex of the right hemisphere. A moderate number of glial cells were in mitotic division and there was perivascular tumor infiltration in nearby regions. This new growth could not be identified further and because it was not recognized in the gross, no transplants were made.

Sarcomas

Extracranial Fibrosarcoma. Mice 14 and 45. In both instances the malignant neoplasms, showing an abundance of reticulin fibers, were entirely extracranial and, therefore, not within the scope of this report. Each seemed to arise from the subcutaneous tissues of the scalp at the sites of the craniotomy wounds and probably arose as the result of the extrusion of the pellet into the burr hole.

Intracranial Fibrosarcoma. Mice 3, 5, 8, 16, 18, 20, 30, 47 and 51. With the exception of mouse 18, which represents a special case, all of the animals of this group developed tumors beneath the intact scalp at the sites of craniotomies. The new growths grew rapidly and caused characteristic deformities of the head (Fig. 8). They all arose intracranially, apparently from the meninges, compressing the adjacent brain tissue. In each instance they mushroomed through the craniotomy opening to their extracranial position beneath the scalp, the intracranial and extracranial portions of the tumor often being connected by a narrow pedicle which was the part that passed through the burr hole (Fig. 9, B.P. 8, B.P. 20 and B.P. 30). In several instances the tumors arose from the meninges in the longitudinal fissure, in others from the cerebral coverings over the right hemisphere. They were always in contact with the benzpyrene pellet, rather well circumscribed and sharply demarcated from the adjacent nervous tissue which they failed to invade.

Microscopically they were composed of mesodermal cells of spindle shape, usually arranged in parallel rows. Nuclear division by mitosis was often present, as were also multinucleated cellular elements. Each tumor had an abundant reticular stroma which was easily impregnated with silver by the Wilder method. In transplants these neoplasms grew rapidly and maintained their microscopic characteristics.

Mouse 18 did not develop a cranial deformity. At necropsy the benzpyrene pellet was found embedded in the subcortex of the right cerebral hemisphere, but no evidence of tumor was visible to the naked eye. Microscopically, however, spindle-shaped cells, many in mitotic division, were present near the pellet and invaded the overlying pia-arachnoid. Although sharply demarcated from the cerebral tissue, these cells invaded the sheaths of parenchymal vessels and also infiltrated the choroid plexus. They produced a fibrillary reticulum which was argentophilic in Wilder preparations. In contrast, therefore, to the other intracranial sarcomas this neoplasm had the earmarks of a primary cerebral sarcoma originating from the perivascular sheaths.

Rhabdomyosarcoma. Mice 6 and 13. Both of these tumors were

entirely extracranial and were recognized as swellings in the temporal region during the lives of the animals. They arose apparently from the temporal muscles and consisted of elongated cells with oval nuclei, large bodies of deeply eosinophilic cytoplasm and interlacing fibrous processes. Scattered throughout the tumor were groups of large oval or round cells with two or more huge vesicular nuclei. Although these cells suggested strongly a striated muscle origin, striations were not demonstrable in their cytoplasm.

Mixed Glioma and Sarcoma

Mouse 1. A tumor mass appeared in the subcutaneous tissues of the scalp in the right parietal region 4 days before this animal died. At necropsy, the extracranial portion of the neoplasm was firm and gray and resembled the extracranial sarcomas. But that part of the neoplasm which was intracerebral was softer in consistency and poorly differentiated from the neighboring brain tissue (Fig. 9, B.P 1). That there were two different tumors became apparent from the microscopic study. The extracerebral tumor consisted of spindle-shaped cells with pale, oval nuclei, each having a single nucleolus. These cells lay in sheets and in whorls and produced a dense reticular stroma which was argentophilic. This was obviously a sarcoma.

The infiltrating intracerebral tumor was of gliogenous origin and consisted of small round cells, with dark homogeneous nuclei, and no reticulin fibers. The cells were scattered helter-skelter in no characteristic fashion. Small foci of necrosis were present but failed to produce any alignment of tumor cells around them. Mitotic figures were present in moderate numbers.

Unfortunately, only the extracranial neoplasm was employed for transplantation. These transplants grew exceedingly rapidly, all of them reaching a minimum size of 1 cm. in approximately 2 weeks. A total of 16 mice were used in four series of 4 animals each, and all of their tumors were sarcomas. Failure to employ the intracerebral tumor for transplantation precluded the opportunity for a more definite diagnosis and hence it must be listed as an unclassified glioma.

Comment

Approximately 50 per cent (24 out of 47) of the mice which had benzpyrene implanted in their brains developed intracranial neoplasms, excluding the two instances of extracranial fibrosarcoma and the two of rhabdomyosarcoma. This incidence compares favorably with that of tumors induced by methylcholanthrene, which was 46 per cent of C_3H mice.[1] Of the 24 benzpyrene-induced intracranial neo-

plasms, half were gliomas. This is in contrast to the apparent ability of pure methylcholanthrene to induce about five times as many gliomas as intracranial sarcomas. The sex of the animals appears to be of no importance in influencing the tumor incidence with benzpyrene; tumor-bearing mice were about equally divided between the sexes.

An effort was made in these experiments to implant the carcinogen rather deeply in the brain tissue, and in four instances where this resulted in the deposition of the pellets within the ventricular system, ependymoblastomas developed. The concept is thereby strengthened that the site of origin of the brain tumor influences its type.

Attention is called to the fact that a medulloblastoma developed in the right cerebral hemisphere, a very unusual site for this type of tumor. This experience, however, was paralleled in the experiments with methylcholanthrene, in which one medulloblastoma also was found in the cerebrum.

There was but little difference in the time required for the development of the gliomas and sarcomas. The average survival time of the animals with tumors of the first variety was 276 days, and of those with sarcomas, 245 days. This difference of a month may be even less significant since it represents the survival time of the animals rather than the appearance time of the neoplasms. In general, gliomas grow somewhat more slowly than sarcomas and, therefore, the survival of animals with tumors of the former type would naturally be longer.

The growth behavior of the gliomas was distinct from that of the sarcomas, as a comparison of Figures 1 and 9 will disclose. Whereas the gliomas infiltrated and faded imperceptibly into the brain tissue, the sarcomas were distinctly circumscribed and sharply demarcated from the nervous parenchyma.

Summary

Pellets of 3, 4-benzpyrene were implanted in the right cerebral hemispheres of 47 C_3H mice of both sexes.

Twenty-eight tumors developed, of which 14 were gliomas; 9 were intracranial fibrosarcomas; 2 were extracranial fibrosarcomas; 2 were extracranial rhabdomyosarcomas; 1 was a mixed glioma and sarcoma. The types of glioma were: astrocytoma, ependymoblastoma, glioblastoma multiforme, medulloblastoma and oligodendroglioma. Four gliomas were unclassified.

The appearance of ependymoblastomas around pellets implanted in the ventricular system lends support to the concept that the *type* of glioma is a function of the *site* of its development.

A significant difference in the survival time of mice with gliomas

and intracranial sarcomas induced with benzpyrene was not found. This is all the more true of the rates of development of these two types of neoplasms.

The macroscopic appearance of the gliomas distinguished them from the intracranial sarcomas. The former were infiltrative and poorly demarcated from the brain tissue; the latter were distinctly circumscribed.

Subcutaneous transplantation of the intracranial tumors was found to be a valuable aid in the study and classification of these new growths.

The present experiments represent the first successful attempts to induce gliomas with benzpyrene.

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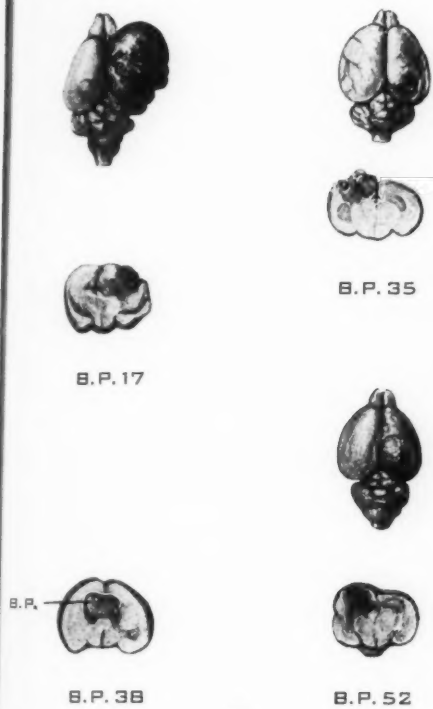
DESCRIPTION OF PLATES

PLATE 115

FIG. 1. *Mouse 17.* Glioblastoma multiforme. Drawing of hemorrhagic, infiltrating tumor in right cerebral hemisphere. *Mouse 35.* Glioblastoma multiforme. Drawing of gray, opaque and partly hemorrhagic, infiltrating tumor in right occipital lobe. *Mouse 38.* Ependymoblastoma. Drawing of tumor surrounding pellet of benzpyrene (B.P.) in ventricle. *Mouse 52.* Medulloblastoma. Drawing of hemorrhagic tumor mass infiltrating the posterior half of the right cerebral hemisphere.

FIG. 2. Mouse 40. Ependymoblastoma. Photomicrograph of tumor in cerebral leptomeninges. Typical rosettes and numerous mitoses are seen. Hematoxylin and eosin stain. × 375.

FIG. 3. Mouse 40. Ependymoblastoma. Photomicrograph of subcutaneous tumor transplant. Hematoxylin and eosin stain. × 375.



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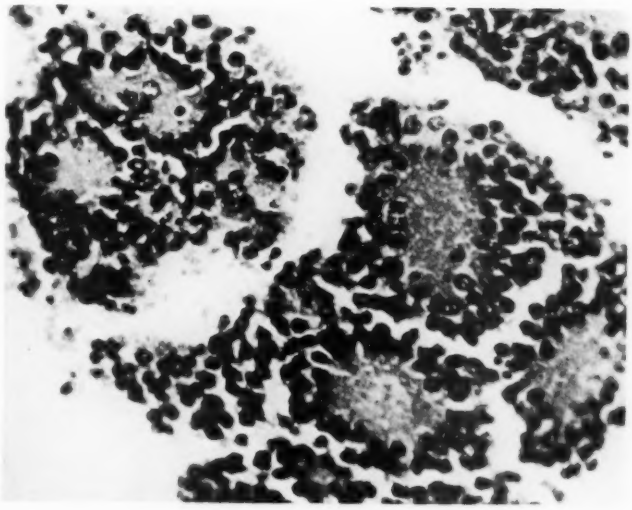

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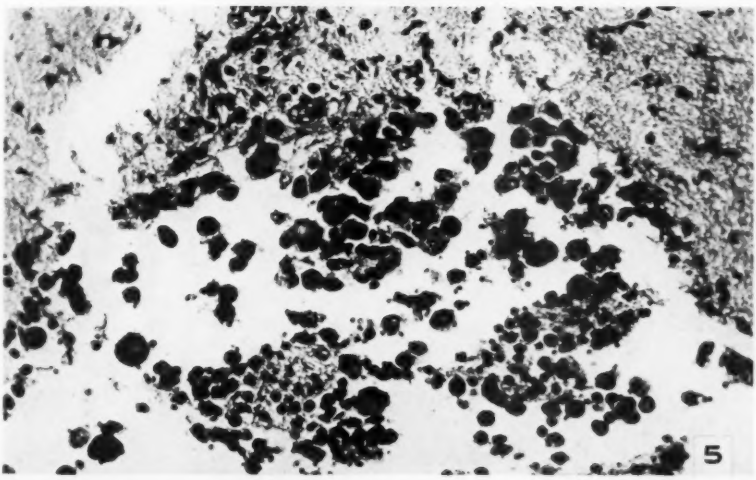
PLATE 116

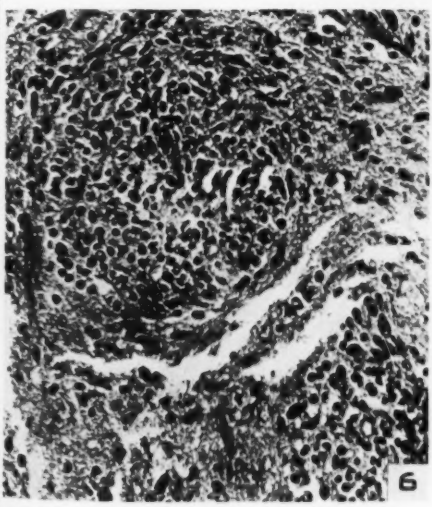
FIG. 4. Mouse 37. Unclassified glioma. Transverse section of brain showing ventricular dilatation and location of tumor in septum pellucidum. Hematoxylin and eosin stain. × 6.

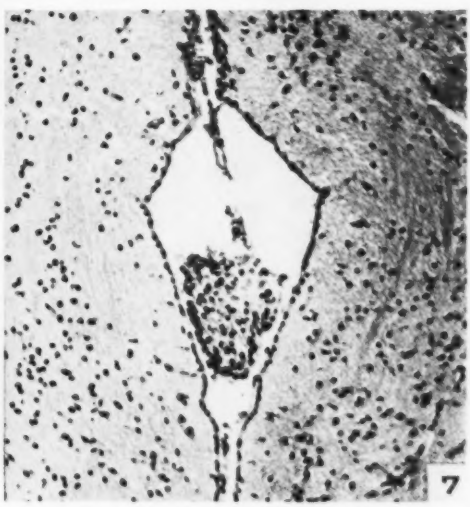
FIG. 5. Mouse 37. Unclassified glioma. Tumor cells in corpus callosum. Hematoxylin and eosin stain. × 200.

FIG. 6. Mouse 37. Unclassified glioma. Tumor nodule stained by the Wilder method, showing absence of reticulin fibers. × 200.

FIG. 7. Mouse 37. Unclassified glioma. Tumor nodule in third ventricle. Hematoxylin and eosin stain. × 100.







Zimmerman and Arnold Brain Tumors Produced with Benzpyrene

Plate 117

Fig. 8. Mouse 47. Characteristic deformity of head due to subcutaneous extension of intracranial fibrosarcoma through burr hole.

Fig. 9. *Mouse 1.* Mixed glioma and sarcoma. The infiltrating cerebral tumor is a glioma; the extracerebral neoplasm is a sarcoma. *Mouse 8.* Intracranial fibrosarcoma. Drawing of sharply circumscribed sarcoma arising in longitudinal fissure and extending extracranially. The ring of tissue around the circumference of the tumor in the upper drawing represents calvarium. *Mouse 20.* Intracranial fibrosarcoma. Drawing of circumscribed tumor in longitudinal fissure. *Mouse 30.* Intracranial fibrosarcoma. Drawing of large tumor arising in longitudinal fissure, compressing adjacent cerebral hemispheres and presenting extracranially. Distinct demarcation from the nervous parenchyma is apparent.

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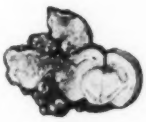
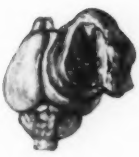


B.P. 1

B.P. 8



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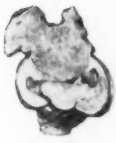

B.P. 30

Zimmerman and Arnold

Brain Tumors Produced with Benzpyrene

A CYTOLOGICAL STUDY OF THE TUBULAR EPITHELIUM IN ACUTE AND CHRONIC CANINE BRIGHT'S DISEASE WITH ESPECIAL REFERENCE TO THE MITOCHONDRIA*

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Though the detailed correlation of the functional and structural characteristics of the tubule of the normal nephron may be still far from adequate, it is fair to state that considerable data concerning both of these aspects of the problem of renal activity are in hand. In the case of nephrons altered by disease, these essential problems have as yet been approached only indirectly from the functional side, and in the morphological problem there is at best only information available concerning the grosser alterations of their configuration.[1]

An extensive literature describes the cellular changes observed in the tubular epithelium in chronic Bright's disease. The greater part of it is concerned with what are called acute "degenerative" lesions as these are observed during varying stages of the development of the renal lesion. There are also descriptions of the regenerative processes which repair these retrogressive lesions. The cells accomplishing this restitution become in the end the only remaining functioning elements of the tubule and so must determine, or be determined by, tubular function. Concerning them, our knowledge can at present be summarized only in terms of an "atypical epithelium," for no cytological detail is available.

One of the most specific and well characterized cytological elements of the normal tubule cell is its mitochondrial apparatus. The present study attempts to describe these structures in the atypical renal epithelium of the kidney in canine Bright's disease.

The mitochondrial constituents of the kidney have been investigated experimentally in numerous acute renal lesions [2] but only one detailed study has been made of a chronic lesion, namely, Oliver's [3] description of the mitochondria in chronic uranium nephritis. In that investigation special emphasis was placed on the relation of the mitochondrial elements to epithelial regenerative activity. As it is essential to obtain absolutely fresh tissue for satisfactory mitochondrial staining, there are no references to similar studies of human renal disease, with the exception of Fahr's [4] description of the mitochondria in hyaline granular degeneration. The mitochondria have likewise not been examined in the naturally occurring kidney diseases of animals where fresh

* Received for publication, January 15, 1943.

material is readily available. Since the tubular epithelial changes in spontaneous renal disease of the dog closely resemble those in human renal disease, the conclusion is tenable that the morphological changes in the mitochondria of both species might be similar, if not identical. The problem in human Bright's disease may therefore be attacked indirectly by study of the animal disease.

With this object in mind, the mitochondrial elements in suitably fixed and stained kidneys of dogs with spontaneous renal lesions were examined and their appearance considered in relationship to the general morphological cellular characteristics as shown by such routine stains as hematoxylin and eosin. In the animal disease, as in human Bright's disease, the cytological changes are pronounced in the proximal convoluted tubule and the present observations are principally concerned with this portion of the nephron.

Although the mitochondria are theoretically well defined cytoplasmic constituents, their exact composition is unknown and their normal function and pathological alterations have been the subject of much controversy. The only specific method for their demonstration is supravital staining with Janus green and similar dyes. Such a procedure was impossible to apply to the dog kidney for technical reasons. It must be recognized that in fixed material other cytoplasmic materials and structures in addition to the mitochondria may take the mitochondrial stain. Exact identification of granular structures in the strict cytological sense therefore becomes difficult, if not impossible, but, in spite of this inexactness, the term mitochondria has been retained for want of any better designation. It is appreciated that conclusions based on the findings in the renal epithelium are of limited application in the general cytological problem of the mitochondria.

Material and Methods

The material studied was obtained from the kidneys of dogs brought to my animal hospital, and includes the acute nephroses in which only the lesions of cloudy swelling, hyaline droplet degeneration, fatty metamorphosis, vascular degeneration and necrosis were found; chronic nephroses such as amyloid contracted kidney; and inflammatory lesions, such as interstitial nephritis,[5] and suppurative nephritis. In the dog, lipoid nephrosis is unknown and glomerulonephritis is exceedingly rare.[5] The kidneys of animals with leptospirosis[6] also offered excellent material for the study of regeneration because of the marked renal epithelial damage that occurs in this infection. In addition, mitochondrial studies were made of the kidneys of four normal dogs.

The clinical status of renal change in each case was determined

by appropriate blood and urine examination. When the condition was apparently incurable or when the owner refused to authorize treatment, the dog was killed by intravenous injection of soluble pentobarbital, as the mitochondria are not altered by this agent.

Necropsies were performed immediately and tissues were fixed in Zenker's fluid-formaldehyde solution and in a 10 per cent solution of neutral formaldehyde U.S.P. Regaud's fluid was used to preserve tissue for mitochondrial staining. Routine sections were stained with hematoxylin and eosin, and frozen sections of formaldehyde-preserved tissue were stained for fats with Sudan III. The mitochondria were stained with Altmann's acid fuchsin and picric acid, Cowdry's acid fuchsin and methyl green, Mallory's phosphotungstic acid hematoxylin and Heidenhain's iron hematoxylin.

Observations

The morphological characteristics of the normal mitochondria of the dog's kidney can be summarized as follows. In the periglomerular portion of the proximal convoluted tubule the mitochondria occur as long, thin, parallel, well stained, rod-like filaments extending from the basement membrane to the supranuclear region of the cell. They are very closely packed, have a uniform thickness with rounded ends and are occasionally somewhat wavy. Many terminate just below the brush border. This appearance is best observed in tubules cut at right angles to the lumina and in longitudinal sections that pass through the axis of the tubule (Fig. 2). If the tubule section is imperfect, the rods appear as round granules of uniform size whose diameter is the same as the width of the individual rod. The terminal portion of the proximal convolution in the dog is characterized by the almost constant presence of a physiological fatty infiltration. In the fat-rich cells the mitochondria are absent or occur as a few small granules interspersed between the crowded fat droplets. In the fat-free cells of this portion of the tubule the mitochondria are similar to those of the periglomerular part of the convolution but are reduced in number. In the thin descending limb of Henle's loop the mitochondria consist of sparse numbers of pale-staining fine granules and thin, short, bacilli-like rods. These usually occupy a perinuclear position and are rarely present in the supranuclear region, which appears as a clear, cap-like, mitochondria-free space. In the thick ascending limb of the loop and the distal convoluted tubule the mitochondria occur as short, stout, homogeneous rods extending from the basement membrane to the supranuclear region. They are paler staining and less densely packed than those in the periglomerular portion of the proximal convolution.

In the collecting tubules the mitochondria are exceedingly sparse and consist of pale-staining, very short, thin, delicate rods and fine granules indiscriminately scattered in the cytoplasm.

The mitochondrial changes in the various renal lesions to be described can be conveniently classified by considering the types of epithelial cells in which they occur. The categories of epithelia that develop in a chronic renal lesion have been previously enumerated [7] and this classification will be used as a basis for the morphological descriptions of the mitochondria.

1. Persisting Epithelium of the Original Renal Type

(*a*) *In Cells Normal in Structure.* The mitochondria showed no change from the normal picture in cells normal in structure.

(*b*) *In Cells Undergoing Acute Regressive Changes* such as cloudy swelling, hyaline droplet accumulation, fatty metamorphosis, vacuolar degeneration and necrosis. A variety of mitochondrial alterations occurred in these degenerative states. In some the mitochondria had lost their long filamentous appearance and fragmented into occasional small fine rods and numerous fine granules whose diameter did not exceed the width of an individual rod. Such cells were frequently swollen and presented the usual characteristics of cloudy swelling (Fig. 3). In other cases the granules were deeply staining, coarse and usually globular, with considerable irregular variation in size and a greater diameter than the width of the normal rod (Figs. 4 and 5). These cells, in hematoxylin and eosin preparations, indicated a more advanced stage of cloudy swelling with large, coarse, eosinophilic granules in a greatly swollen cytoplasm. Granules of all these types diffusely filled the cytoplasm, showing no definite cellular orientation. Material having all the staining characteristics of mitochondria also assumed "droplet" form. The droplets, large, deeply staining, spherical and with smooth contours, varied considerably in size and were often as large as a nucleus (Fig. 6). They likewise evidenced no definite cellular distribution and occasionally occurred in the tubular lumina. The droplets appeared in sections stained with hematoxylin and eosin as typical "colloid droplet" formations. In cells that were definitely necrotic the granules were agglutinated into large, irregular masses of mitochondrial material that were more heavily concentrated in the basal or distal portions of the cell and which frequently fused with similar conglomerated material in adjacent cells. In vacuolar degeneration the vacuoles themselves contained no mitochondrial material although fine granules and short rods were commonly present in the intervacuolar cytoplasm (Fig. 7). Mitochondria were usually absent

in degenerating cells containing fat similar in appearance to that seen in the physiological infiltration of the cells of the terminal proximal convolutions.

(*c*) *In Epithelium Undergoing Progressive (Hypertrophic or Hyperplastic) Change.* The filamentous mitochondria of the hypertrophic periglomerular proximal convolutions in chronic renal lesions were definitely lengthened although there was little, if any, increase in their width (Fig. 8). In striking contrast there was no increase of the normally sparse number of mitochondria in the hyperplastic collecting tubules (Fig. 9).

(*d*) *Hypertrophic Cells in Which Degenerative Change Has Secondarily Occurred.* The mitochondrial alterations, namely, fine and coarse granules, droplets and agglutinated masses, in these large cells were similar to those seen in normal cells undergoing regressive changes (Fig. 10).

2. Newly Formed Regenerated Epithelium

Among the kidneys in all of the conditions studied there were found all stages in the regeneration of epithelium. Numerous mitotic figures were present and there was great variation in the amount of mitochondrial material in these cells. Some cells in mitosis contained large numbers of granular mitochondria and in others these elements were completely absent. In addition, dividing cells could be found whose mitochondrial content varied in a quantitative manner between these extremes, so that a series could be arranged beginning with those containing many granules and continuing with cells almost completely absent (Fig. 1). The majority of the mitotic figures showed abnormalities in the arrangement and distribution of their chromatin. Hyperchromatic (Fig. 1, h and j) and hypochromatic (Fig. 1, d) figures were common, as well as pyknosis (Fig. 1, c) and rhexis of chromosomes (Fig 1, e and i), as these terms are used by Politzer.[8] Giganticism also was observed even in the earliest prophases of division (Fig. 1, a and b).

As the cells of tubules with regenerated epithelium do not show a constant fixed cytological type, various stages in the maturity of the new-formed cells could be identified. Tubules with early regeneration of epithelium often consisted of a bare membrana propria with several large, oval, hyperchromatic nuclei that projected into the lumen and which were covered with a thin, indistinct cytoplasmic layer. In these cells mitochondria were completely absent or at best only an occasional granule was present (Fig. 11). The epithelium of other tubules with more advanced regenerative changes consisted of many large nuclei lying in an undifferentiated, cytoplasmic, syncytial mass. These often

assumed the appearance of "giant cells" and consisted of from 6 to 15 nuclei in a mass of cytoplasm that extended into and more or less filled the tubular lumen. In the syncytial masses and "giant cell" complexes, only a sparse number of small, granular mitochondria were present and in some of these tubules, mitochondria were completely absent (Figs. 12 and 13). The cytoplasm of these cells, however, usually contained a fine, dust-like material that took the mitochondrial stain very faintly.

In kidneys with acute degenerative lesions but in which there was no interstitial proliferation of connective tissue, the new cells gradually took on, with the passage of time, the appearance of the adult epithelium and eventually could not be differentiated from the original structures. In these cells, the nuclei decreased in size, lost their vesicular appearance and regained their usual polarity in relation to the tubular lumen; the cytoplasm became more distinct and evidenced cellular differentiation. In addition, there was an early development of fine granules and short rods until the mitochondria characteristic of the original cell type appeared (Figs. 14 and 15).

In kidneys with chronic Bright's disease, namely, amyloid contracted kidney and chronic interstitial nephritis, the atypical regenerated epithelium in tubules in areas of interstitial fibrosis persisted as such and never regained the structure of the normal adult cell, so that these distorted tubules were lined with an irregular layer of epithelium often several cells thick, whose oval nuclei showed no orientation in regard to the axis of the tubule. In the atypical cells of these tubules the mitochondria never assumed their normal rod-like structure but consisted of scattered small granules in sparse numbers. This persistence of large groups of distorted tubules with atypical epithelium and scanty mitochondria was noted even in those damaged kidneys where, judging by the clinical evidence of adequate renal elimination, functional compensation had occurred. In such instances the convoluted tubules outside the scarred areas were hypertrophied and hyperplastic and possessed an entirely normal mitochondrial pattern.[7]

3. *Epithelium of the Preceding Categories That Has Undergone Simple Atrophy*

The mitochondria of atrophic epithelium, whether compressed and distorted, or flattened, were similar. They consisted of small, fine, pale-staining granules and short, thin rods that were often more heavily concentrated around the nucleus (Fig. 16). Even in tubules whose lumina were obliterated and whose membranae propriae had blended with the surrounding connective tissue, the epithelial cells contained

few persisting mitochondrial granules. These atrophic cells rarely showed degenerative change, such as large granule forms, droplets, or agglutinated masses.

Discussion

In general, it may be said that the morphology of the mitochondria is apparently correlated with the general histological appearance of the tubular epithelium, so that if one is familiar by means of special stains with the mitochondrial changes peculiar to the various epithelial alterations, it is possible to predict with a fair degree of certainty the microscopical mitochondrial morphology by the study of even a simple routine preparation.

As has been stated, most of the experimental investigations relating to the renal mitochondria have been concerned with their reactions in acute renal lesions, and these have been adequately summarized by Cowdry.[2] The mitochondrial changes in the degenerative epithelial lesions (Figs. 3 to 7) in my series of spontaneous renal disease closely approximate those that have been previously described in experimental acute renal damage.

The derivation of the hyaline droplets which occur in the renal epithelium in a variety of conditions deserves special comment as these have been interpreted as being of exogenous origin by some, *i.e.*, as protein absorbed from the tubular lumen, and of endogenous origin, arising from intracellular mitochondrial material, by others. In my material, the hyaline droplets have the tinctorial properties of mitochondrial substance and in the same cell all forms, from small granules to large droplets, may be seen together (Fig. 6). The histological data suggest, therefore, that the droplets consist, in part at least, of mitochondrial material, or that this material enters into their composition and that droplets may arise by swelling, solution and fusion of the granules.

According to Cowdry,[2] an increase of mitochondrial material is relatively rare and has been reported in the thyroid, in the islets of Langerhans during diabetes, in the kidney following the administration of phloridzin, and in actively regenerating muscle and cartilage cells. In the cases of compensated chronic nephritis of this series the mitochondrial rods of the hypertrophic periglomerular proximal tubules are definitely lengthened with no increase in width, so that the total amount of mitochondrial material within each cell is augmented (Fig. 8). This increase becomes considerable when the exact mathematical dimensions, which have been calculated from measurements of microdissected specimens, are considered,[8] inasmuch as the hypertrophic

proximal convolution is not only wider and has a greater volume but may be almost twice as long as the normal.

Addis and Oliver [9] summarized the facts concerning the regenerative processes of the tubular epithelium in human renal disease. Essentially, the reparative epithelial changes are similar in Bright's disease of man and dog and in experimental investigations with bichromate salts, corrosive sublimate, or uranium nitrate. The epithelium which has escaped the destructive action proliferates by mitosis and possibly by amitosis. In my material, abnormal mitotic figures were the rule. The series which may be set up of cells with mitotic figures, some with many granules and others with none, apparently indicates a continuing decrease with eventual disappearance of the mitochondria during the course of abnormal renal regenerative activity (Fig. 1). The mechanism of this phenomenon may be as follows: With the first division of the cell the number of mitochondria distributed to each daughter cell is approximately half that of the mother cell and with each rapidly succeeding division the mitochondria are further reduced so that the definitive regenerated cell contains very few, if any, granules. Although the studies of Lewis and Lewis [10] of tissue cultures show wide variations in the amount or number of the mitochondria in proliferating chick embryo cells, Cowdry,[11] also working with chick embryos, came to the general conclusion that the number, shape and arrangement of mitochondria during mitotic division are essentially the same as in nondividing cells. This discrepancy with what I have described can be explained by the fact that in my material regenerative activity followed definite cellular injury, as contrasted with the mitotic activity of the normal cells of the chick embryo, and that in the chronic lesions it proceeded under the conditions of a chronic disease which precluded the re-establishment of the original normal status.

The finely granular, extremely pale-staining, dust-like material (Figs. 12 and 13) in the regenerated epithelium can be interpreted either as representing further fragmentation of the persisting mitochondria or as an early stage in the formation of new mitochondrial substance. The former supposition appears more likely as occasional tubules were seen lined in part by epithelium of mature appearance in which mitochondrial substance was completely absent, suggesting that this granular material had gone into solution and disappeared. This action might be considered as an indication of continuing cellular damage exerted by the toxic or infectious agent responsible for the initial renal lesion, and that the agent was still active in its nephrotoxic effect on the newly formed immature epithelium.

For years, cytologists have been interested in the doctrine of mito-

chondrial continuity,[12] in respect to whether the mitochondria in proliferating cells arise in the cytoplasm *de novo*, or only through multiplication by division of pre-existing units. While the evidence presented here is far from incontrovertible, it seemed more likely that the mitochondria of the latter generations of regenerated cells arose in the cytoplasm *de novo* as a result of the cells' metabolism and not through division of pre-existing units.

It is generally known, as Thorel [13] first demonstrated, that in many epithelial degenerations in the kidney the regenerated cells, though at first of an embryonic type, later assume a form which differs in no degree from the adult type of the original renal cells. However, the early proliferation of connective tissue in chronic nephritis [3] apparently prevents the regenerated epithelium from reaching maturity and thus the epithelium never regains the mitochondrial characteristics of the adult cell. Addis and Oliver [9] suggested that a similar mechanism may operate in the kidneys of man with chronic Bright's disease. Certainly in chronic canine Bright's disease, the "atypical" epithelium has a permanently reduced mitochondrial content, and in this respect closely resembles the earlier generations of immature epithelium that are found in the repair of transient degenerative renal lesions. Whether this inability of differentiation to the original cell type is due to the persisting toxic effect that produced the original retrogressive change in the epithelium or to abnormalities in environment and nutrition that have developed in the altered renal tissue during the course of the chronic disease is a speculative question. This problem is further complicated by the possibility of a genetic factor, for it is certain that the atypical epithelium arises in large part by atypical karyokinesis.

Architecturally, *i.e.*, in the configuration and number of their nephrons, the clinically compensated and decompensated kidneys of chronic canine Bright's disease may be similar, although in the tubules of the latter are found characteristic cellular structural alterations. In a previous study [7] it has been shown that from the morphological viewpoint, the mitochondrial elements of the proximal convolution are essentially normal in the adequately compensated organ and considerably disarranged in the decompensated kidney. The difficulty of ascertaining the functional state of a kidney by examination of the routinely stained section is well known. A definite indication can be obtained as to the state of compensation or decompensation in chronic canine Bright's disease, however, by the application of stains for the mitochondrial apparatus. This is true, of course, only when fresh tissue is immediately preserved in suitable fixatives, for poor or late fixation simulates the same disintegration of the mitochondrial elements that

is found in the decompensated kidney associated with bona fide disease processes.

The tubule of the nephron in chronic Bright's disease is lined with an epithelium that differs from the normal not only in its general cellular characteristics but equally so in the intimate cytological structures of its cells. Speculations as to the existence of possible correlated disturbances of its function would be premature, but if one accepts the general proposition of correlation of structure and function, associated disarrangements of the latter might be suspected.

Conclusions

1. A cytological study of the kidneys of dogs with spontaneous acute and chronic Bright's disease indicates specific alterations of the mitochondrial elements in the tubular epithelium, associated with the regressive, progressive and regenerative changes.

2. The morphological mitochondrial pattern is closely correlated with the general histological appearance of the tubular epithelium as determined by routine stains.

3. As the general morphological aspect of the tubular epithelial changes in human and canine Bright's disease are essentially the same, it is suggested that the mitochondrial alterations in both species may be similar.

I wish to thank Dr. Jean Oliver for his guidance and aid in this work.

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[Illustrations follow]

DESCRIPTION OF PLATES

KEY TO PLATE 118

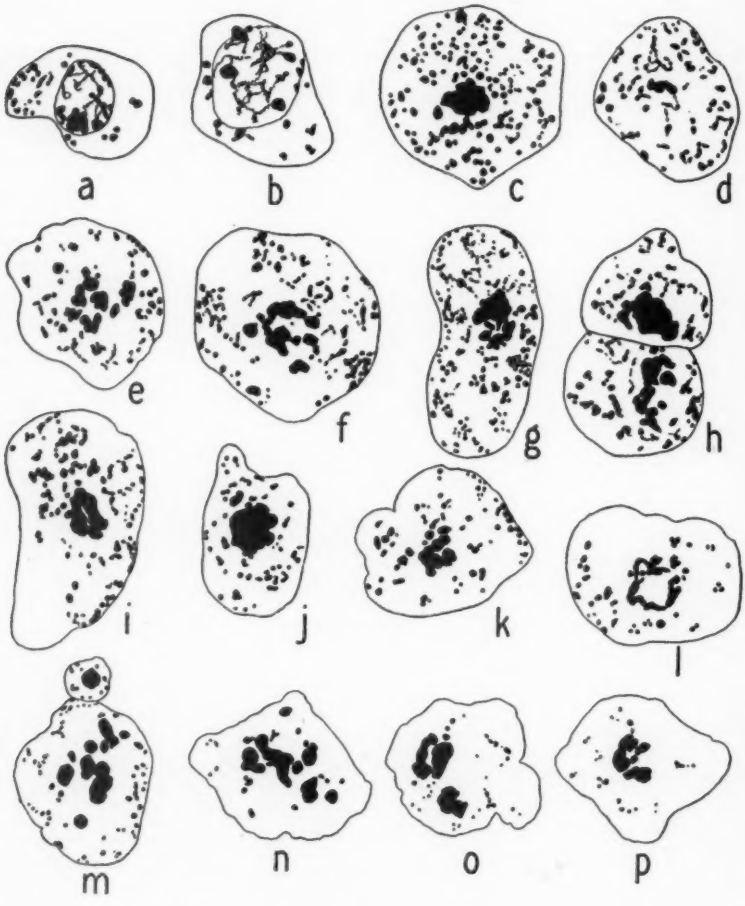
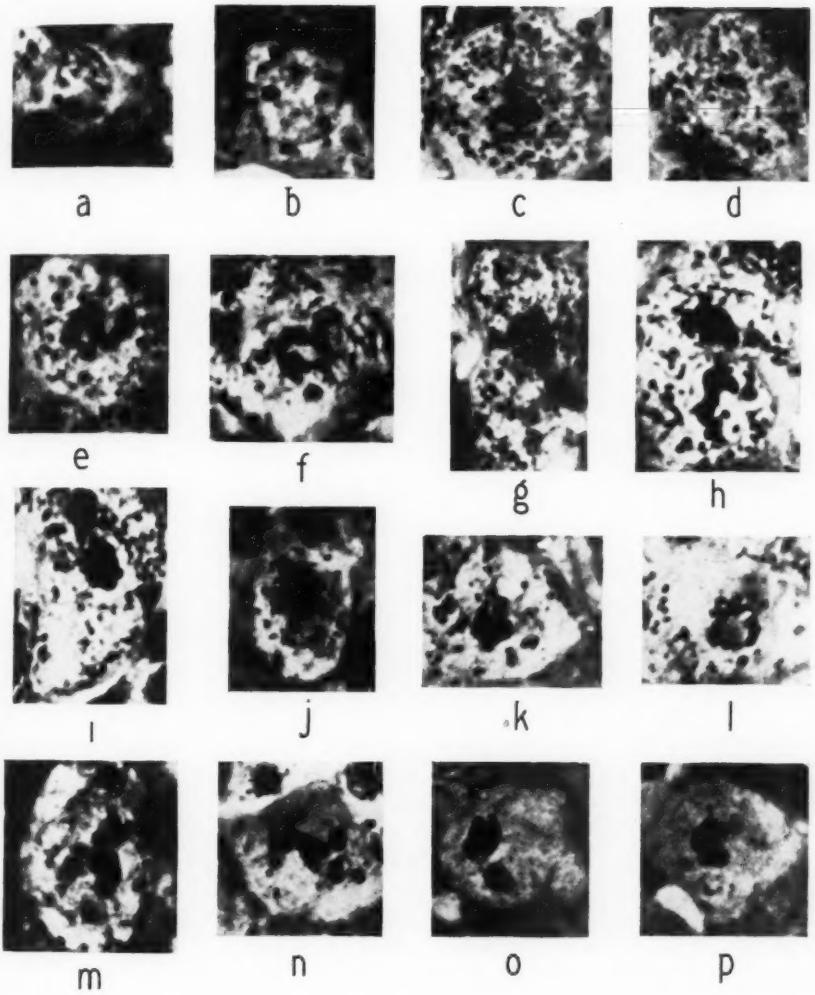


FIG. 1. a to p, mitotic figures from regenerating renal epithelium in canine Bright's disease. a, c, d, f, j and p, chronic interstitial nephritis, iron hematoxylin stain; b, e, k, m and d, subacute interstitial nephritis, Mallory's phosphotungstic acid hematoxylin stain; g, h, i, l, n and p, subacute nephrosis, iron hematoxylin stain. × 1500.



Bloom

1

Tubular Epithelium in Canine Bright's Disease

PLATE 119

FIG. 2. Case 709. Chronic interstitial nephritis. Cross section of proximal convolution in the periglomerular mass. The persisting tubular epithelium is entirely normal. The mitochondria appear as long, densely packed, filamentous rods. Iron hematoxylin stain. × 1000.

FIG. 3. Case 887. Chronic interstitial nephritis. A periglomerular proximal convolution shows various degenerative disarrangements in the mitochondria. At the left, dissolution of the mitochondrial rods and vacuole formation are seen; in the middle portions the filaments have broken up into fine granules; at the right, these granules are agglutinated into an irregular mass. Mallory's phosphotungstic acid hematoxylin stain. × 1000.

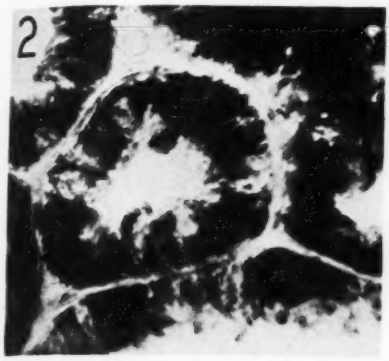
FIG. 4. Case 5935. Leptospirosis. A longitudinal section of a periglomerular proximal tubule in which the normal rod structure has disintegrated and the cytoplasm is filled with large, coarse, deeply staining granular mitochondria. Mallory's phosphotungstic acid hematoxylin stain. × 1000.

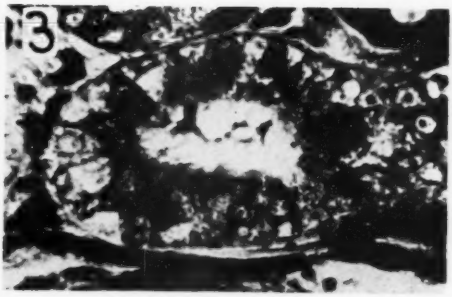
FIG. 5. Case 5592. Acute interstitial nephritis. Sections of two periglomerular proximal convolutions with advanced regressive changes. Coarse granular mitochondria are present in fewer numbers as compared to Figure 4. Mallory's phosphotungstic acid hematoxylin stain. × 1000.

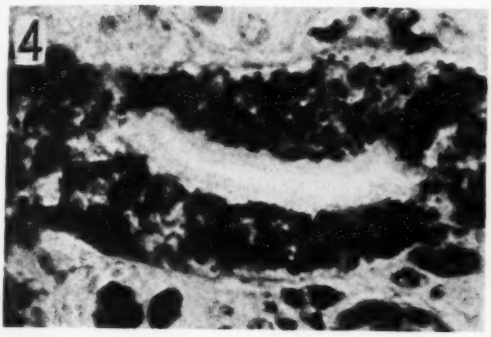
FIG. 6. Case 887. Amyloid contracted kidney. A cross section of a periglomerular proximal convolution from the same case as shown in Figure 10. The cytoplasm and tubular lumen contain numerous smaller and larger droplets of material with the staining reactions of mitochondria. Mallory's phosphotungstic acid hematoxylin stain. × 1000.

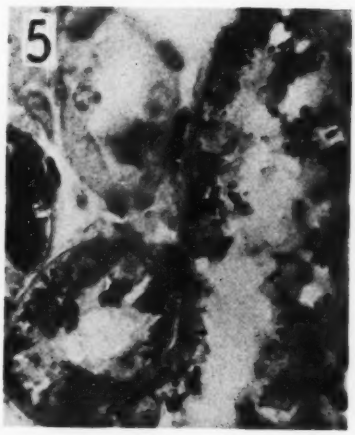
FIG. 7. Case 6457. Leptospirosis. Cross sections of periglomerular proximal convolutions showing the marked cytoplasmic vacuolation and sparse mitochondrial granules in the intervacuolar cytoplasm. Mallory's phosphotungstic hematoxylin stain. × 800.

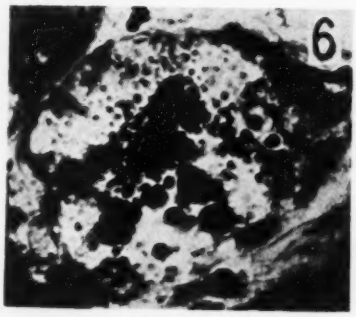
FIG. 8. Case 709. Chronic interstitial nephritis. A greatly hypertrophied proximal convolution from the periglomerular mass of the same kidney as shown in Figure 2. Except for increase in size, the cellular configurations are entirely normal. The mitochondria are correspondingly increased in number and in length. Figures 2 and 8 are shown at the same magnification and are therefore directly comparable. Mallory's phosphotungstic acid hematoxylin stain. × 1000.











Bloom

Tubular Epithelium in Canine Bright's Disease

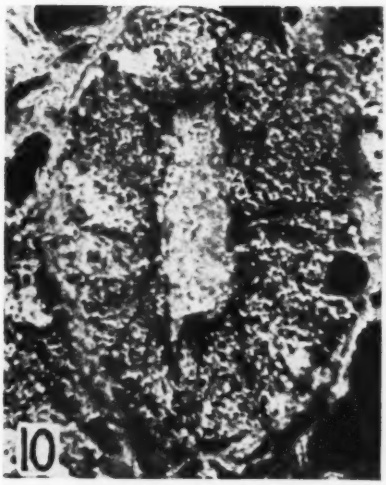
PLATE 120

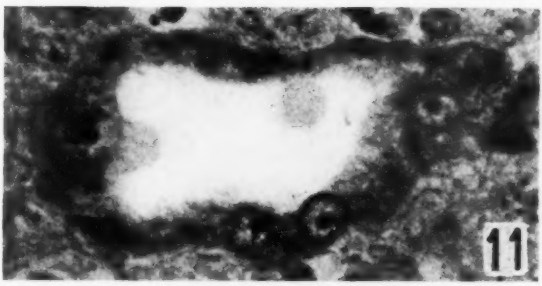
FIG. 9. Case 709. Section of a markedly hypertrophic collecting tubule from the same kidney as shown in Figure 8. In these large epithelial cells there is no increase in the mitochondria, which are originally scanty in these tubules. Iron hematoxylin stain. × 1000.

FIG. 10. Case 887. Amyloid contracted kidney. Cross section of a hypertrophic periglomerular proximal convolution in which the normal rods are absent and the mitochondria are fragmented into fine, paler staining granules. Mallory's phosphotungstic acid hematoxylin stain. × 1000.

FIG. 11. Case 5935. Leptospirosis. An atypical tubule with several regenerated cells, in one of which there is a mitotic figure. The nuclei are large and oval and the cytoplasm indefinite and scanty. There is practically complete absence of mitochondrial granules, although finely granular, dust-like material is present. Mallory's phosphotungstic acid hematoxylin stain. × 1000.

FIG. 12. Case 5935. Leptospirosis. A later developmental stage in which the cells assume a giant-cell-like appearance. Very few pale-staining mitochondrial granules are present in addition to the dust-like granular material that very faintly takes the mitochondrial stain. Mallory's phosphotungstic acid hematoxylin stain. × 1000.





Bloom Tubular Epithelium in Canine Bright's Disease

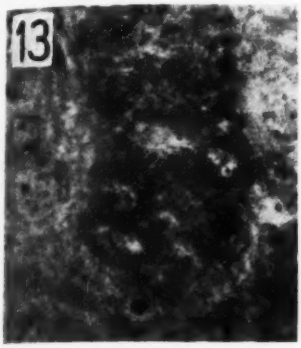
PLATE 121

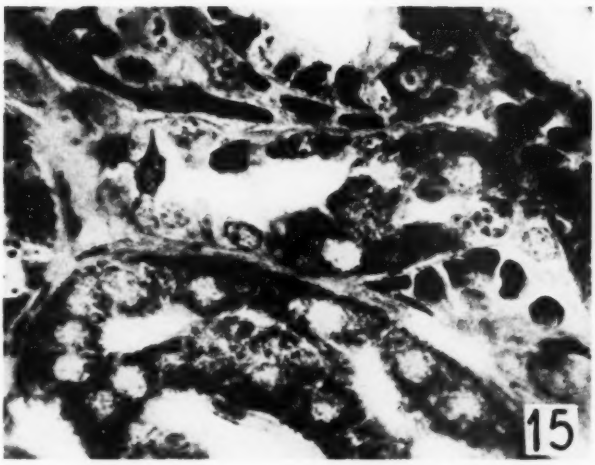
FIG. 13. Case 5935. Leptospirosis. Another tubule from the same kidney as shown in Figure 12, in which there are a greater number of mitochondrial granules and more dust-like, finely granular material. Mallory's phosphotungstic acid hematoxylin stain. × 1000.

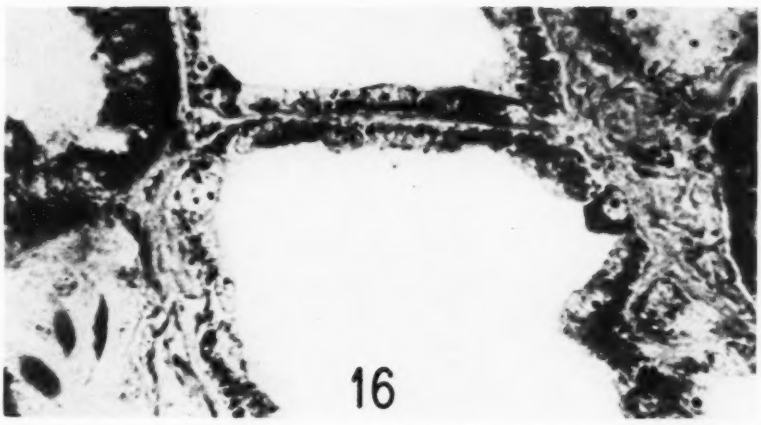
FIG. 14. Case 5592. Acute interstitial nephritis. Lying diagonally are longitudinal sections of two tubules, the cells of which are beginning to assume a more adult appearance. In these there is a perinuclear distribution of small, granular mitochondria while the remaining immature cells are lacking granules. Compare with the deep-staining, coarse granules of the degenerated epithelium in the right lower corner. Mallory's phosphotungstic acid hematoxylin stain. × 1000.

FIG. 15. Case 5592. Acute interstitial nephritis. The cells in the left portion of the upper middle tubule are similar to those of Figure 14, with only a few cells showing perinuclear granular mitochondria. In the portion of the same tubule to the right the cytoplasm of the cells of more mature configuration is markedly increased and contains numerous closely packed granules. These latter cells are considered a later stage in epithelial regeneration. The tubule below is completely lined by similar cells. Mallory's phosphotungstic acid hematoxylin stain. × 1000.

FIG. 16. Case 200. Chronic interstitial nephritis. Widely dilated tubules with flattened atrophic epithelium in which fine granules and short rods are present. Mallory's phosphotungstic acid hematoxylin stain. × 1000.







Bloom Tubular Epithelium in Canine Bright's Disease

NECROTIZING ARTERITIS IN DOGS RELATED TO DIET AND RENAL INSUFFICIENCY

V. Evidence for a Dietary Factor*

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In recent publications from this laboratory [1-4] it has been shown that acute necrotizing arterial lesions affecting principally the large elastic arteries (aorta, pulmonary artery, endocardium of the left auricle) occur with regularity following the production of renal insufficiency in dogs that have been fed a standard low protein diet. The manner in which renal insufficiency was produced—uranium nitrate, mercuric chloride, or bilateral nephrectomy—did not influence the incidence of these arterial lesions. Only two factors seem to be necessary for their production: (1) a factor associated with renal insufficiency, and (2) a dietary factor. The first factor has received ample confirmation from the work of Winternitz and his co-workers [5, 6] who have produced somewhat similar lesions in dogs by injecting various extracts of different organs in dogs with both kidneys removed, by ligation of both renal arteries and by total and subtotal obstruction of both ureters. The work of Goldblatt,[7] showing that renal ischemia severe enough to cause renal insufficiency leads not only to hypertension but also to a necrotizing arteriolitis such as is encountered in human cases of malignant hypertension, also confirms the factor associated with renal insufficiency. Neither of these groups of workers, however, has found it necessary to control the diet of its experimental animals. In our experience to date, diet seems to play a definite rôle. It is the purpose of this paper to describe in detail the preparation and feeding of the standard diet and to present the data that incline us toward the belief that a dietary factor is fundamentally associated with the lesions.

Methods

All of the dogs were healthy adult mongrels. Pups and obviously old dogs were excluded. They were brought into the kennel from routine local sources and were kept in "isolation" for 2 weeks before being placed in the main dog room. Both during the 2 weeks' period of "isolation" and during their subsequent stay in the main dog room they were kept in individual cages (on shavings that were changed three

* Aided by a grant from the John and Mary R. Markle Foundation.
Received for publication, January 18, 1943.

times per week). They had free access to water at all times and, unless otherwise indicated, they were fed the regular kennel ration which has varied from time to time. The major portion of this ration has at times consisted of cooked table scraps selected from refuse at the University dining hall cafeteria. At other times the kennel ration has consisted chiefly of uncooked bones with adherent fat and meat. Occasional feedings of Purina dog chow have been used when other food was not available. Temperature and humidity in the animal quarters are controlled throughout the year. During the course of these experiments there have been no outbreaks of distemper or other known infectious diseases.

The principal variables in the experiments reported in this and the following papers are four in number: (1) diet, (2) renal insufficiency, (3) "plasma alteration" and (4) time. The methods for controlling these variables have been described in previous publications.[1-4] Since the emphasis in this paper is on diet, the preparation and feeding of the standard diet is given in detail.

The "standard diet" fed most of the animals in the experimental group consisted of: calves' liver (raw wet weight), 32 parts; cane sugar, 25 parts; corn starch, 25 parts; butter, 12 parts; and cod liver oil, 6 parts. One gm. of McCollum-Simmonds salt mixture [8] and 5 gm. of kaolin were thoroughly mixed with each day's diet. Enough tomato juice was added to make a paste of which each gram contained 3 calories. The diet was fed in amounts to furnish 75 calories per Kg. per day. Essentially the diet is a low protein diet with 7 per cent of its caloric value derived from protein, 50 per cent from carbohydrate and 43 per cent from fat.

In actual practice the total number of dog diets for about 1 week (5 to 10 days extremes) was calculated and a batch of food to supply this number of kilogram-dog-days was prepared as follows:

Cane sugar	19.2 parts per 100
Corn starch	19.2 parts per 100
Kaolin	4.0 parts per 100
Salt mixture	0.8 parts per 100

After this dry material was thoroughly mixed, the following were added:

Liver (finely ground in meat chopper)	24.0 parts per 100
Tomato juice	18.4 parts per 100

Again the mixture was stirred and the following added:

Cod liver oil (commercial)	4.8 parts per 100
Butter (melted but not boiled)	9.6 parts per 100

These last two were thoroughly stirred into the mixture to form a homogeneous paste which was kept in the ice box at 36° to 38° C.

Each day's food for each dog fed the standard diet was weighed out into individual pans and placed in the cage, customarily between 2 and 4 p.m. Any food left the following morning was weighed and complete records were kept of dietary consumption. Rarely a dog (less than 1 out of 10) refused to eat the diet, hence all dogs were placed on this standard diet for a few days (usually 3) to determine that the diet would be eaten before control blood-level studies were obtained. Dogs made hypoproteinemic were fasted for 1 week before being returned to the standard diet which was fed while the daily exchanges were being made. There was no interruption of the diet in any other dogs.

"Plasma alteration," when employed, depended on one of three technics: (1) induction of hypoproteinemia, (2) plasma injections and (3) citrate injections. Hypoproteinemia was produced by plasmapheresis; hyperproteinemia, by repeated intravenous injections of homologous plasma (the anticoagulant was tri-sodium citrate, 1.25 cc. of a saturated aqueous solution—about 0.8 gm. per 100 cc. of donor's blood); and citrate injections consisted of repeated intravenous injections of an equivalent amount of tri-sodium citrate added to 100 cc. of 0.9 per cent sodium chloride.

Renal insufficiency was produced in one of three ways: (1) uranium nitrate (uranyl nitrate, Merck, lot S-.7497) injected into the subcutaneous tissues of the groin in 0.5 per cent aqueous solution; (2) mercuric chloride (Baker's analyzed, lot 6939) injected into the external jugular vein in 0.1 per cent aqueous solution; and (3) bilateral nephrectomy performed with aseptic technic.

Following the production of renal insufficiency the dogs usually ceased eating from 1 to 10 days later and 2 to 10 days before they died. The dogs that survived renal injury varied to some extent, but most of them continued to eat the diet each day of their survival period. The data related to dietary consumption *after* renal insufficiency have been analyzed, but no significant differences related to this factor could be detected; the important factor seemed to be the duration on the standard diet *before* renal insufficiency was produced.

Nonprotein nitrogen was determined by duplicate micro-Kjeldahl analysis. The protein precipitant used in separating protein from nonprotein nitrogen was 10 per cent trichloracetic acid.

The experimental animals were kept under close observation, were sacrificed with ether if they were obviously moribund and were necropsied promptly after death. Routine sections were taken from the

following sites: mucous membranes of mouth, injection site in groin, heart [three sections: (a) left ventricle, mitral valve and left auricle—this section included the circumflex branch of the left coronary artery; (b) right ventricle, pulmonic valve and pulmonary artery; and (c) interventricular septum, aortic valve and base of aorta], aorta and any of its larger branches that showed gross change, lungs (at least one section from the hilus region), spleen, liver, gallbladder, pancreas, adrenal, kidneys, bladder, prostate and testis in males, ovary and uterus in females, stomach, small intestine (usually including a Peyer's patch), colon, mesenteric lymph nodes, thymus, thyroid and parathyroid, hypophysis, brain, eye, rib, and femoral bone marrow. The procedure was varied to include any gross lesions. Tissue from these sites was fixed in a 4 per cent solution of formaldehyde and in Zenker's solution. The Zenker's fixed tissues were sectioned at 7 μ and stained routinely with hematoxylin and eosin. Special stains were used in selected cases.

Experimental Observations

The data which form the basis for this paper have been abstracted from two series of experiments: (1) a series primarily for a study of arterial lesions in which most of the dogs were fed the "standard diet," and (2) a control series (an outgrowth from the first series) which was designed to study the effect of sodium citrate on heavy metal injury and in which practically all of the dogs were fed the "kennel diet." Most of the control data come from this second series of experiments.[9] In Table 1 of the following paper [10] the data on the first series of dogs are given in some detail, and the subgrouping of these dogs—by diet, period on diet, method of production of renal injury, and "plasma alteration"—has been indicated by numbers in the last column. These subgroup numbers appear in some of the tables of this paper. This method of presentation of the data enables one to check illustrative examples of the type of experiment referred to in the tables of this paper. The difference between the numbers listed in the latter and the number of examples given in Table 1 of the following paper [10] represents the number of dogs in the "control" or "sodium citrate" series.

Table 1 groups all of the dogs together to substantiate the effect of diet upon the incidence of arterial lesions. Of the 97 dogs which consumed an unknown amount of a variable *kennel diet* for an indefinite period of time before being subjected to renal insufficiency by uranium nitrate or mercuric chloride, only 5, or 5.2 per cent, developed arterial lesions. The incidence of arterial lesions was about four times as great

TABLE I

Influence of Diet, Method of Production of Renal Insufficiency, and "Plasma Alteration" on the Incidence of Arterial Lesions

Diet	Renal insufficiency produced by	"Plasma alteration"	Number of dogs	Number with arterial lesions	Per cent positive	Subgroup number*
Kennel	Uranium nitrate	None	22	0	0	1
		Citrate injections	49	2	4	2
	Mercuric chloride	None	11	1	9	3
		Citrate injections	15	2	13	4
	Total		97	5	5	
"Standard"	Uranium nitrate	None (4 wks. of diet)	5	0	0	5
		None (10 wks. of diet)	5	5	100	6
		Citrate injections	3	0	0	7
		Plasma injections	6	6	100	8
		Hypoproteinemia	2	2	100	9
	Mercuric chloride	None (6 wks. of diet)	3	2	67	10
		Plasma injections	3	2	67	11
	Bilateral nephrectomy	None (20 wks. of diet)	3	2	67	12
		Plasma injections	3	3	100	13
	Total		33	22	67	
Lean Meat	Uranium nitrate	Plasma injections	3	0	0	14
	Mercuric chloride	Plasma injections	1	0	0	15
	Total		4	0	0	

* These subgroup numbers correspond with those in Table I of the following paper.[10]

in the groups injected with mercuric chloride as in the groups injected with uranium nitrate; and four of the five instances of arterial lesions occurred in dogs that had received citrate injections. A much larger series of animals would be necessary to establish the significance of these statements; but it is significant that only 5 of the entire group developed arterial lesions, and it is worth noting that of the 2 dogs with lesions in subgroup 2 (uranium nitrate plus citrate injections) both had evidence of chronic renal damage—in one, chronic glomerulonephritis, and in the other chronic pyelonephritis. Since this is the first time that we have referred to arterial lesions in control dogs it seems advisable to document them.

A brief summary of the clinical data on these 5 dogs is given in Table II and the anatomical distribution of the lesions is listed in Table III. Typical lesions are illustrated in Figures 1 to 4. As near as I can tell, these lesions are identical with those described and illustrated in previous publications. The first change is edema and swelling of the intercellular tissue of the large elastic arteries (ascending aorta, pulmonary artery, endocardium of the left auricle) usually

TABLE II

Summary of Experimental Data on "Control" Dogs with Arterial Lesions

Dog number	Sex	Estimated age	Body weight	Diet*	Renal† injury produced by	Survival interval	Terminal N. P. N.	Remarks
		(*yrs.*)	(*Kg.*)			(*days*)	(*mg. per 100 cc.*)	
40–72	F	4	24.8	K. D.	Hg 2.0	4	330	Dog used as donor for 18 months
40–88	M	1	17.5	P. D. C.	Hg 3.0	10	523	
B–31			19.2	K. D.	Hg 3.0	10	320	
K–20	M		11.5	K. D.	U 5.0	14	605	Chronic pyelonephritis
K–42		12	22.0	K. D.	U 5.0	11	292	Chronic glomerulonephritis

* P.D.C. = Purina dog chow; K.D. = kennel diet.

† Hg 3.0 = mercuric chloride, 3.0 mg./Kg.; U 5.0 = uranium nitrate, 5.0 mg./Kg.

TABLE III

Anatomical Distribution of Arterial Lesions *

Dog number	Ascending aorta	Coronary arteries	Left auricle	Pulmonary artery	Other arteries
40–72			+	++†	Acute periarteritis of myocardial arterioles (+)†
40–88		+	+†	+	Mesenteric (+) and left subclavian (+)
B–31	++	+	+++	+++	
K–20	+	+	++		
K–42	+†	+	+++	+++	

* The lesions have been graded as follows: +++ = gross lesion over 1 cm. in maximum diameter; ++ = gross lesion less than 1 cm. in maximum diameter; + = lesion discovered in microscopical sections.

† Microscopical illustration.

near the internal elastic membrane. This is rapidly followed by outpouring of fibrin, red blood cells and leukocytes. The predominant recognizable leukocyte is the polymorphonuclear neutrophil, but the majority of the leukocytes are most accurately described as "cells with distorted nuclei." Nuclear fragmentation and other necrotic changes are marked. The lesion remains confined beneath intact endothelium until it has attained considerable size, then it may break through the endothelial covering with ensuing thrombus formation. In the advanced lesions the elastic framework is fragmented and partially lysed, but in the early lesions the elastic tissue shows only swelling. Sometimes calcium is deposited in the lesions. For further details the reader is referred to previous publications.[1-4]

In contrast to the control group on kennel diet is the group of 33 dogs which were fed the *"standard diet"* for 4 weeks or longer before

TABLE IV

Effect of Diet upon the Incidence of Arterial Lesions following Renal Injury

Dietary group	Number of animals	Number with arterial lesions	Per cent positive
Standard diet	25	22	88.0
Kennel diet	97	5	5.2

a comparable degree of renal injury was produced (by uranium nitrate in 21, by mercuric chloride in 6, and by bilateral nephrectomy in 6). Typical arterial lesions occurred in 22 of this group, an incidence of 67 per cent (Table 1). If the 5 dogs which were fed the standard diet for only 4 weeks (subgroup 5) and the 3 dogs which received citrate injections (subgroup 7) are excluded, the incidence becomes 22 of 25 positive, or 88 per cent (Table IV). The lesions both grossly and histologically have always corresponded to the interval between the production of renal insufficiency and death (4 days to 11 months). In addition to the usual acute necrotizing lesions which have been described and illustrated in previous publications [1-4] and which are essentially identical to those described, there have been 2 dogs with aneurysms (both near the mouth of the innominate artery) and 4 dogs with healing or healed lesions. Of the 3 dogs which did not show lesions, 1 (dog 40-83, subgroup 12) died 3 days after bilateral nephrectomy and showed early changes but no definite necrosis, another (dog 42-2, subgroup 10) was fed the standard diet for only 6 weeks before renal injury was produced, and for the third (dog 41-89, subgroup 11) there were no circumstances which might explain the lack of lesions.

The general statement that with the same degree of renal injury

about 90 per cent of the dogs on the standard diet developed arterial lesions while the incidence of similar lesions in dogs fed the regular kennel ration was about 5 per cent holds; but the detailed data must be analyzed further in order to correlate them with previously published results.[1-4] Several facts emerge from such an analysis.

First, the duration of maintenance on the standard diet before renal insufficiency was produced is important (Table V). While none of 5 dogs fed the standard diet for only 4 weeks before renal injury was produced (subgroup 5) showed lesions, 9 of 11 dogs kept on the standard diet for 6 weeks or longer (subgroups 6, 10 and 12) showed lesions after comparable renal injury. Of the two failures in this group, 1 (dog 42-2) consumed the diet for only 6 weeks and the other (dog 40-83) lived only 3 days after bilateral nephrectomy. These

TABLE V

Influence of Length of Time on Standard Diet upon the Incidence of Arterial Lesions following Renal Injury

Time on standard diet	Subgroup numbers*	Number of animals	Number with arterial lesions	Per cent positive
Short (3 to 6 weeks)				
With plasma alteration	8, 11, 13, 9	14	13	92.9
Without plasma alteration	5	5	0	0
Long (6 weeks to 6 months)				
Without plasma alteration	6, 10, 12	11	9	81.0

* These subgroup numbers correspond with those in Table I of the following paper.[10]

studies are being continued in an attempt to define "optimum conditions" for the production of the arterial lesions.

Second, injections of citrated plasma obtained from healthy donor dogs accelerate or augment the production of the arterial lesions. This was the procedure used at the time these lesions were first observed; [1] and many experiments bear out this second point. Eleven of 12 dogs fed the standard diet for 3 to 5 weeks while they were receiving daily intravenous injections of homologous plasma (injections that averaged about one-third of the total plasma volume of the recipient and thus amounted in all to about five to six times the total plasma volume of the recipient, subgroups 8, 11 and 13) had typical arterial lesions when they died in "uremia" 5 to 17 days after the production of renal insufficiency. Two dogs fed the standard diet for 3 and 5 weeks respectively while they were made hypoproteinemic by plasmapheresis (subgroup 9) also had typical arterial lesions. This incidence is to be contrasted with that of no arterial lesions in 5 dogs, fed the standard diet alone for the same period (subgroup 5, Table V).

Third, with "optimum conditions" tentatively defined as repeated intravenous injections of donor's plasma for 3 to 5 weeks followed by the subcutaneous injection of 5.0 mg. of uranium nitrate per Kg., all 6 dogs fed the standard diet had lesions while none of 4 dogs fed a lean meat diet had lesions (Table VI).

The fourth point does not pertain directly to diet but may have a bearing on the problem as a whole. It grew out of control experiments on the data presented in the preceding paragraph, experiments designed to test the effect of the anticoagulant used in making the injections of donor's plasma.[9] This anticoagulant was tri-sodium citrate, 1.25 cc. of a saturated aqueous solution (about 0.8 gm.) per 100 cc. of donor blood, and over 90 per cent of this was contained in the plasma that

TABLE VI

Influence of Diet under "Optimal" Conditions for the Production of Arterial Lesions*

Dietary group	Subgroup numbers†	Number of animals	Number with arterial lesions	Per cent positive
Standard diet	8	6	6	100.0
Lean meat diet	14, 15	4	0	0

* "Optimal" conditions are tentatively defined as repeated intravenous injections of donor's plasma for 3 to 5 weeks followed by 5.0 mg. of uranium nitrate per Kg.

† These subgroup numbers correspond with those in Table I of the following paper.[10]

was injected into the recipient. When saline plus citrate is injected intravenously instead of plasma plus citrate (the amount of citrate and total volume of fluid being kept constant) not only do the arterial lesions not develop but the dogs are protected against a lethal dose of uranium nitrate. Twelve of 13 (92 per cent) control dogs (without citrate) succumbed in 9 to 13 days to 5.0 mg. of uranium nitrate per Kg.; 13 of 14 (93 per cent) experimental dogs (with citrate) survived the same dose of heavy metal. This protective action was independent of diet. It is noteworthy that the "saline and citrate" did not prevent urinary, blood chemical and anatomical evidence of renal injury.[9] Despite these definite evidences of renal injury, none of these experimental animals showed any evidence of healed arterial lesions when they were subsequently sacrificed. This fourth point can be stated very simply and directly: citrated homologous plasma augments the production of the arterial lesions; citrated saline protects against those following the injection of uranium nitrate. (Experiments to date have failed to show that citrate affords any protection against mercuric chloride injury[11] and it could not be expected to protect against bilateral nephrectomy.)

Discussion

The chief point indicative of a dietary factor that has emerged from these studies is that with the same degree of renal injury about 90 per cent of the dogs fed the standard diet (22 of 25) have developed necrotizing arterial lesions, while only 5 per cent of 97 "control" dogs consuming an unknown amount of a variable kennel ration have developed similar lesions (Table IV). At present I have no definite evidence as to the nature of the dietary factor; *i.e.*, whether it is (1) a deficiency, (2) a toxic factor, or (3) an imbalance. Studies are under way at the present time in an attempt to isolate and define the dietary factor.

Necrotizing arterial lesions in dogs have been produced in other laboratories, but it is not certain that all of these lesions are identical. Goldblatt,[7] using bilateral subtotal renal arterial constriction, has produced principally necrotizing arteriolitis. This localization—especially in the visceral arterioles—may be related to the hypertension that precedes and accompanies the arteriolar changes. The few scattered determinations of blood pressure which have been made in connection with these experiments have not yielded consistent results, but there is ample evidence that hypertension is not a necessary precursor to the lesions in the large elastic arteries. An occasional arteriolar change has been seen and illustrated in the myocardium,[3] lungs, and submucosa of the stomach (never in the liver, pancreas, adrenals, or kidneys). By far the major damage to the arterial system seen in my dogs, however, has been in the large elastic arteries; in particular, the endocardium of the left auricle, the pulmonary artery, and the ascending limb of the arch of the aorta, including the mouth of the innominate artery.

Winternitz and his colleagues[5, 6] have found "hemorrhagic" and "necrotizing" lesions in various organs and tissues following the production of renal insufficiency in dogs in a variety of ways, including bilateral nephrectomy and subtotal ureteral obstruction. Reference to the following paper[10] indicates that I, too, have seen many lesions that could be described as "hemorrhagic" and/or "necrotizing." These lesions (stomatitis, gastroenteritis and pancreatic fat necrosis) are far less dependent on diet than are the arterial lesions which are referred to in this paper. If only lesions of the arterial tree are considered, it is not clear that the New Haven group has encountered the same lesions that I have. Most of the lesions which that group has illustrated have been in muscular arteries or in arterioles. In the few lesions in large elastic arteries which they illustrate, hemorrhage and necrosis without much leukocytic response characterize the lesions.

Polymorphonuclear neutrophils and "cells with distorted nuclei" dominate the lesions in my dogs, and, from the available descriptions, the lesions seem to be much more extensive than are those of Winternitz *et al.* Quantitative factors may be involved, for there are many factors in common between the arterial and other lesions (see following paper [10]) produced in this laboratory and those produced in the New Haven laboratory.

The aortas and pulmonary arteries of some of the "control" dogs fed the kennel diet and especially of the dogs fed the lean meat diet (subgroups 14 and 15) showed rather striking edema of the inner third of the media and the intima, and a few showed "bubble elevation" of the endothelial lining as if by edema; but hemorrhage, leukocytic reaction and necrosis were absent. Some of the lesions resemble those reported by Hueper [12] following the intravenous injections of macromolecular substances.

These differences in the arterial lesions produced by various procedures in various laboratories have been referred to in some detail, for none of these other laboratories has found it necessary to control the diet of its experimental animals. The work of the New Haven and Cleveland groups is stressed in particular, for there is one factor common to all of the recently published results [5-7] from those laboratories as well as from this laboratory, namely, renal insufficiency. This seems to be *sine qua non* so far as the production of the necrotizing arterial lesions in question is concerned. If a dietary factor is involved, it is one that manifests itself only in the presence of renal insufficiency. The "standard diet" which my dogs have consumed is adequate, for dogs have been maintained on it in good clinical condition with no loss of weight for over a year. A borderline deficiency might be involved but this could probably be defined more accurately as depletion of a "reserve store" of some hypothetical factor. Quantitative factors are almost certainly involved, for under "optimum conditions for the production of the lesions," a shift to a lean meat diet completely alters the incidence of the lesions (Table VI). It is possible that the dogs on the lean meat diet stored some substance necessary for the maintenance of elastic arteries in sufficient amount to "buffer" them against the damaging effect of renal insufficiency.

Further speculation is unwarranted at this time. The immediate question is: Is a dietary factor involved? Our experience to date indicates that if normal adult dogs are fed the "standard diet" for 2 months or longer before being subjected to renal insufficiency by either mercuric chloride or uranium nitrate or by bilateral nephrectomy, 9 out of 10 (if they live 4 days or longer after the renal injury) will show

a necrotizing process in the large elastic arteries—in particular in one or more of these three sites: (1) endocardium of the left auricle, (2) pulmonary artery and (3) ascending limb of the arch of the aorta including the mouth of the innominate artery.

Summary

Acute necrotizing arterial lesions affecting principally the large elastic arteries (aorta, endocardium of the left auricle, pulmonary and coronary arteries) have been produced with regularity in dogs by controlling two factors: (1) renal insufficiency and (2) diet. Only the first of these factors has been confirmed in other laboratories.[5-7] In this paper the preparation and feeding of the "standard diet" which has been used in this laboratory are described in detail. The data that indicate that a dietary factor is fundamentally associated with the lesions can be summarized as follows:

1. The strongest evidence for a dietary factor is that with the same degree of renal insufficiency, 88 per cent (22 of 25) of the dogs fed the "standard diet" have had typical arterial lesions while similar lesions have occurred in only 5.2 per cent of 97 "control dogs" fed the regular kennel diet. Arterial lesions in the "control dogs" are described and illustrated for the first time.

2. The period of feeding the "standard diet" before the production of renal insufficiency is important. Data obtained thus far indicate that a minimum of about 8 weeks is necessary, but this period can be greatly shortened by repeated intravenous injections of citrated plasma obtained from healthy donor dogs.

3. While citrate and homologous plasma augment the production of the lesions (shorten the period of "standard diet" feeding), saline citrate solution prevents the lesions and protects the dog against death after a dose of uranium nitrate that proved lethal to 12 of 13 controls.

4. The method by which renal insufficiency has been produced has not influenced the incidence of the arterial lesions except in so far as it has affected the survival period (the longer this period the more advanced the lesions).

5. Experiments are still in progress to determine "optimum conditions" for the production of these arterial lesions. Under "optimum conditions," tentatively defined as daily injections of homologous plasma six times per week for 3 to 5 weeks before the subcutaneous injection of 5.0 mg. of uranium nitrate per Kg., all 6 dogs fed the "standard diet" during this period showed typical arterial lesions while none of 4 dogs fed a diet of lean meat developed lesions.

All dogs in both the control group (kennel diet) and in the experi-

mental group (standard diet) that have developed arterial lesions have been subjected to renal injury, and the lesions have always corresponded, both grossly and histologically, to the interval between the production of renal insufficiency and death. Hence, if a dietary factor is involved in these arterial lesions, it is a dietary factor that manifests itself only in the presence of renal insufficiency. Before speculating on the nature of the dietary factor or on the manner in which renal insufficiency influences a dietary factor, it is first necessary to determine whether a dietary factor is involved. The data to date indicate that it is.

The author is indebted to Dr. G. L. Donnelly and his assistants, Mr. W. H. Meroney and Mr. C. J. Ross, for examining the heart and large blood vessels of many of the dogs.

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[Illustrations follow]

DESCRIPTION OF PLATE

PLATE 122

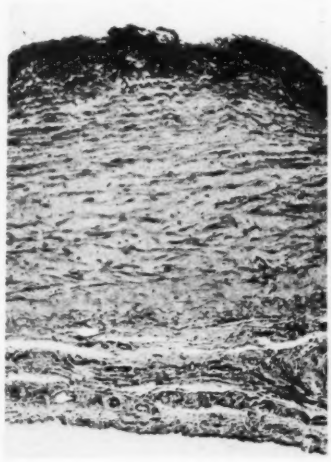
Necrotizing arterial lesions in "control" dogs. All sections are stained with hematoxylin and eosin.

FIG. 1. Dog K–42. Aorta. Acute necrotizing aortitis of inner portion of wall. Edema of remainder of media. × 50.

FIG. 2. Dog 40–72. Pulmonary artery. Acute necrotizing arteritis. × 140.

FIG. 3. Dog 40–88. Acute necrotizing left auriculitis. × 140.

FIG. 4. Dog 40–72. Acute periarteritis of myocardial arteriole. × 140.



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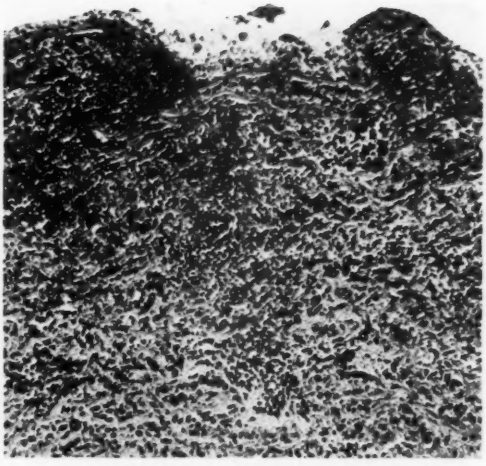

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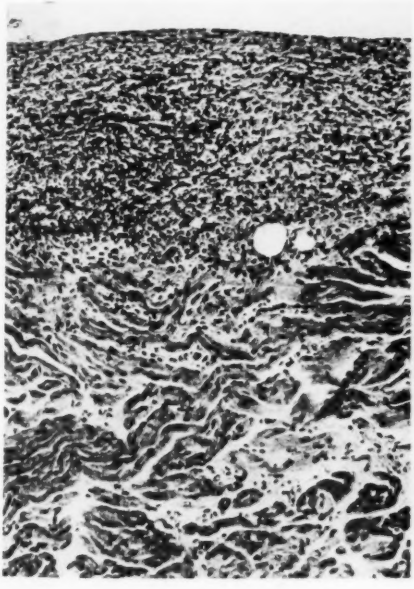

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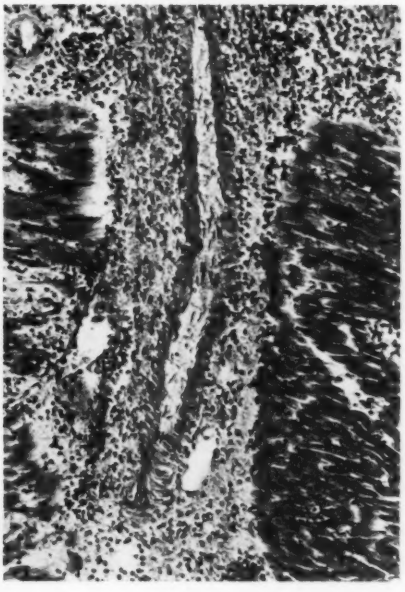

4

Holman Experimental Necrotizing Arteritis. V

NECROTIZING ARTERITIS IN DOGS RELATED TO DIET AND RENAL INSUFFICIENCY

VI. Associated Lesions: Stomatitis, Gastroenteritis and Pancreatic Fat Necrosis*

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In the preceding paper [1] and in previous publications [2-5] some of the anatomical changes that follow the production of renal insufficiency in dogs have been presented. In reports thus far, attention has been focused on the changes in the arterial system, especially in the large elastic arteries such as the aorta, endocardium of the left auricle, pulmonary and coronary arteries. In essence these changes amount to an acute necrotizing arteritis not unlike that encountered in human cases of rheumatic arteritis and periarteritis nodosa, but no claim of identity has been made. The data to date have supported the belief that these changes are related to a dietary factor that manifests itself only in the presence of renal insufficiency.

Lesions in three other anatomical sites have occurred in these dogs with sufficient frequency to relate them to at least one part of the experimental procedure (renal insufficiency). These sites and the corresponding lesions are: (1) mouth—stomatitis; (2) gastrointestinal tract—gastritis, enteritis and colitis; and (3) peripancreatic fat—pancreatic fat necrosis. It is the purpose of this paper to describe these lesions and to try to interpret them in the light of the various experimental procedures. The experimental data afford evidence (1) that "mercurial" stomatitis and gastroenteritis are not due to mercury as such but to the renal insufficiency caused by the heavy metal, and (2) that classical examples of acute inflammation can be caused by an "internal irritant."

Methods

The methods are the same as those described in the preceding paper.[1]

Experimental Observations

The data in this paper are restricted to the 57 dogs on which complete necropsies were performed. The pertinent data on these dogs are given in Table 1. The same method of grading lesions has been used throughout: 3 plus = gross lesion over 1 cm. in maximum diameter; 2 plus = gross lesion less than 1 cm. in maximum diameter; 1 plus =

* Aided by a grant from the John and Mary R. Markle Foundation.
Received for publication, January 18, 1943.

TABLE I

Summary of Experimental Data

Dog no.*	Sex	Estimated age	Body weight at time of renal injury	Diet†	Period on diet before renal injury	Renal injury produced by‡	Plasma alteration§	Survival interval‖	Maximum N. P. N.	Lesions in				Subgroup no.
										Arteries¶	Mouth	Gastrointestinal tract	Peripancreatic fat	
		(*yrs.*)	(*Kg.*)		(*weeks*)			(*days*)	(*mg./100 cc.*)					
41–94	F	1	3.9	Ken.		U 5.0		6	446	–	+	+	–	1
42–27	F	4	4.3	Ken.		U 5.0		6	552	–	+++	–	–	
41–92	F	5	6.3	Ken.		U 5.0		9	388	–	+++	–	–	
41–93	F	1	4.4	Ken.		U 5.0		11	610	–	+++	+	++	
41–91	M	1	8.3	Ken.		U 5.0		28[S]	160	–	–	–	–	
40–78	F	2	6.2	Ken.		U 5.0	Citrate	3[S]	86	–	–	–	–	2
40–75	F	5	6.9	Ken.		U 5.0	Citrate	6[S]	288	–	–	–	–	
40–70	F	1	7.0	Ken.		U 5.0	Citrate	9[S]	495	–	++	–	–	
40–58	F	2	7.2	Ken.		U 5.0	Citrate	12[S]	642	–	++	–	–	
40–65[D]	F	4	20.3	Ken.		Hg 2.0		7	403	–	+	–	–	3
41–97	F	2	14.8	Ken.		Hg 2.0		9	531	–	–	–	–	
40–66[D]	F	4	20.1	Ken.		Hg 2.0		10[S]	112	–	–	–	–	
40–72[D]	F	4	24.8	Ken.		Hg 2.0		10	523	++	+++	+	++	
39–35[D]	M	6	26.0	Ken.		Hg 2.5		3	194	–	++	–	–	
39–36[D]	M	5	23.0	Ken.		Hg 2.5		3	178	–	++	++	–	
39–47[D]	M	4	18.4	Ken.		Hg 2.5		3	311	–	+	–	–	
39–37[D]	M	6	22.0	Ken.		Hg 2.5		4	244	–	++	–	+	
39–39[D]	M	6	19.6	Ken.		Hg 2.5		4	207	–	+	–	–	
40–88	M	1	17.5	Ken.		Hg 3.0	Citrate	4	330	+	++	++	++	4
41–96	F	1	12.0	Ken.		Hg 3.0	Citrate	11	692	–	+++	–	–	
40–53	F	1	7.4	Stan.	4	U 5.0		10	531	–	+++	–	–	5
40–54	F	1	9.4	Stan.	4	U 5.0		12	448	–	++	+	–	
40–51	M	1	7.8	Stan.	4	U 5.0		13	337	–	+	+	++	
40–57	M	1	9.2	Stan.	4	U 5.0		21	457	–	–	+	–	
39–33	F	2	8.2	Stan.	4	U 5.0		122[S]	151	–	–	–	–	
42– 7	M	1	4.0	Stan.	10	U 5.0		10	348	+++	+++	+	++	6
42– 8	F	1	6.8	Stan.	10	U 5.0		10	442	+++	+++	+	–	
42– 5	F	2	6.8	Stan.	12	U 5.0		16	363	++	+++	–	–	
40–68	F	5	3.9	Stan.	12	U 5.0		35[S]	162	+[H]	+[H]	–	–	
40–79	F	1	4.5	Stan.	16	U 5.0		7	544	+++[A]	–	++	–	
	F	2	8.1	Stan.	4	U 5.0	Citrate	160[S]	116	–	–	–	–	7

39–46	F	3	8.1	Stan.	4	U 5.0	Citrate	160[S]	116	−	−	−	−	7
39–48	M	2	9.5	Stan.	4	U 5.0	Citrate	244[S]	49	−	−	−	−	
39–47	M	1	9.0	Stan.	4	U 5.0	Citrate	900[S]	75	−	−	−	−	
39–34	M	2	4.4	Stan.	4	U 5.0	Pl. inj.	8	297	+++	−	+	−	8
39–40	M	1	6.5	Stan.	3	U 5.0	Pl. inj.	11	582	+++	+++	+	−	
39–45	F	2	7.3	Stan.	4	U 5.0	Pl. inj.	14	617	+++	+	+	−	
39–43	F	3	4.7	Stan.	5	U 5.0	Pl. inj.	15	438	+++	+++	+	−	
40–50	F	1	4.8	Stan.	4	U 5.0	Pl. inj.	17	573	++[H]	+++	+	−	
39–28	F	4	5.1	Stan.	5	U 5.0	Pl. inj.	345[S]	92	+++[H]	−	−	−	
38–24	F	3	4.5	Stan.	6	U 3.0	Hypo.	15	386	+++	+++	+	−	9
39–49	M	2	4.8	Stan.	3	U 5.0	Hypo.	15	391	++	+++	+	−	
42– 3	F	3	5.3	Stan.	6	Hg 3.0		4	289	+++	+++	+++	−	10
42– 4	F	1	3.5	Stan.	6	Hg 3.0		5	374	+	+++	−	−	
42– 2	F	2	5.7	Stan.	6	Hg 3.0		6	436	−	+++	−	++	
40–80	F	2	5.8	Stan.	4	Hg 3.0	Pl. inj.	6	466	+++[A]	+++	+++	−	11
40–63	F	2	9.2	Stan.	4	Hg 3.0	Pl. inj.	7	394	+++	+++	+++	++	
41–89	M	2	8.0	Stan.	4	Hg 3.0	Pl. inj.	12[S]	181	−	+++[H]	+	−	
40–83	F	1	6.3	Stan.	23	B. N.		3	348	−	+	+	++	12
40–85	F	1	7.1	Stan.	23	B. N.		4	308	+	+	−	−	
40–84	M	3	7.4	Stan.	19	B. N.		5	244	++	++	++	−	
40–52	F	2	7.3	Stan.	4	B. N.	Pl. inj.	5	710	+	+	+	++	13
42– 1	F	1	5.6	Stan.	6	B. N.	Pl. inj.	6	538	++	+++	+	++	
40–77	F	1	5.2	Stan.	4	B. N.	Pl. inj.	7	295	+	+++	++	−	
40–60	M	1	6.3	L. M.	4	U 5.0	Pl. inj.	10	638	−	+++	−	−	14
40–59	F	2	5.8	L. M.	4	U 5.0	Pl. inj.	13	782	−	+	+	−	
40–64	F	3	4.9	L. M.	4	U 5.0	Pl. inj.	180[S]	239	−	−	−	−	
40–67	F	1	4.3	L. M.	4	Hg 2.5	Pl. inj.	81[S]	59	−	−	−	−	15

* Superscript D in subgroup 3 indicates that dog was used as a donor for a year or longer before being subjected to renal insufficiency.

† Ken. = kennel diet; Stan. = "standard diet"; L.M. = lean meat diet.

‡ U 5.0 = uranium nitrate, 5.0 mg./Kg. subcutaneously; Hg 3.0 = mercuric chloride, 3.0 mg./Kg. intravenously; B.N. = bilateral nephrectomy.

§ Citrate = intravenous injections of sodium citrate; Pl. inj. = intravenous injections of citrated plasma obtained from "donor" dogs; Hypo. = hypoproteinemia by plasmapheresis.

‖ Superscript S indicates that the dog was sacrificed on this day after renal injury was produced. Numbers without "S" indicate period after renal injury at which dog died.

¶ Superscript H = healing or healed; superscript A = aneurysm.

lesion discovered in microscopical sections. The largest lesion encountered served as the basis for this grading. For purposes of completeness and comparison, the arterial lesions discussed in the preceding paper [1] have been listed in this table.

Stomatitis

More data are available on the mouth lesions, for they can be followed from day to day. The method by which renal insufficiency was produced has influenced the time of appearance of the lesions. When mercuric chloride and bilateral nephrectomy were used for this purpose, early lesions have been observed as soon as 36 hours after the renal injury and typical gross lesions have been found when the dog died in the next 36 hours, *i. e.*, 3 days after the production of the renal injury (*cf.* dogs 39–35 and 40–83). When uranium nitrate was used as the nephropathic agent, mouth lesions did not appear before the 4th day and sometimes did not appear until 2 or 3 days before the animal died on the 14th to 17th day after the uranium nitrate was injected. Healing or healed lesions have been observed in 2 dogs that survived the renal injury.

In the majority of cases the mouth lesions have occurred in three locations. The first lesions are usually found on the mucous membrane of the upper lip opposite the alveolar ridge of the premolar teeth on either side. Careful daily examination of this area reveals the first change as an area of slightly dilated capillaries. The mucous membrane over the area is dry and within a short time becomes delicately "sanded" (as if by fibrin). Within 24 hours after these changes definite necrosis with the appearance of patches of grayish yellow exudate are evident. These patches frequently have a thin reddish margin of dilated capillaries, and small hemorrhages have occurred in some of the patches. This same series of events occurs at the other two sites in the oral cavity, namely, the mucous membrane of the cheeks about 1 cm. posterior to the angles of the mouth and on the under surface of the distal half of the tongue near the lateral margins. In advanced cases the necrotic patches on the cheeks have extended forward to join the posterior spread of the lesions of the upper lip, but even in these cases the mucous membrane of the lower lip escapes. The average lesion found at necropsy measures about 12 mm. in diameter (Fig. 1).

By the time the lesions make their appearance the breath is usually fetid, but the odor due to the lesions is ofttimes overshadowed by the odor of "uremia." In these terminal stages there is much drooling of mucoid saliva, which frequently is blood-tinged.

Microscopical examination of the lesions confirms the gross impression that the earliest changes occur beneath intact epithelium. Congestion and edema with margination and emigration of polymorphonuclear neutrophilic leukocytes and outpouring of fibrin have all been observed beneath intact squamous epithelium (Figs. 2 and 4). The majority of the sections show advanced changes with massive polymorphonuclear infiltration, obvious necrosis, and marked bacterial growth on the surface. Even in these advanced cases it is surprising how frequently remnants of the squamous epithelium are recognizable (Figs. 3 and 5). In other words, even the advanced lesions give evidence that ulceration with subsequent invasion of the tissues by mouth organisms was not the first change. Bacterial stains have shown the usual mouth organisms, including spirochetes and fusiform bacilli in some of the sections. In the healing and healed lesions, monocytic removal of débris, granulation tissue becoming progressively less vascular and more fibrous with time, and re-epithelization of the surface have all been observed. The changes are insignificant in the oldest healed lesion.

The incidence of these mouth lesions is much the same in the various groups of experimental animals and is much more independent of diet than are the arterial lesions described in the preceding paper.[1] Their occurrence by method of production of renal insufficiency, by diet and by various "plasma alterations" is indicated in Table II. Forty-two of the 57 dogs (74 per cent) had mouth lesions. All 6 dogs subjected to bilateral nephrectomy, regardless of "plasma alteration," had the lesions, thus excluding the possibility that the lesions are due to heavy metal. Only one group of animals is of interest for the absence of lesions. None of the 3 dogs that received intravenous injections of sodium citrate before being injected with uranium nitrate (39–46, 39–47 and 39–48, subgroup 7) developed mouth lesions. Other published results [6] confirm this finding that sodium citrate protects dogs against uranium nitrate injury. The factor responsible for these mouth lesions remains obscure just as it does in human cases of mercurial stomatitis.

Gastroenteritis

The lesions in the gastrointestinal tract have been divided into gastric (present in 18 of the 57 dogs, or 32 per cent), small intestinal (present in 11 of the 57 dogs, or 19 per cent), and large intestinal (present in 6 of the 57 dogs, or 11 per cent). In all three locations the lesions are essentially similar and are characterized grossly by hemorrhage. The size and distribution of the hemorrhagic lesions in the gastrointestinal tract have varied markedly. In the stomach they

TABLE II

Influence of Method of Production of Renal Insufficiency, Diet and "Plasma Alteration" on the Incidence of Lesions

Renal insufficiency produced by	Diet	"Plasma alteration"	No. of dogs	Number with lesions in							Subgroup no.*
				Mouth	Gastrointestinal tract				Peripancreatic fat	Arteries	
					Stomach	Small intestine	Colon	Total			
Uranium nitrate	Kennel	None	5	4		2		2	1		(1)
		Citrate injections	4	2							(2)
	"Standard"	None (4 wks. of diet)	5	3	1	1	1	3	1		(5)
		None (10 wks. of diet)	5	4	3	1	1	3	1	5	(6)
		Citrate injections	3								(7)
		Plasma injections	6	4	4	2	1	5		6	(8)
		Hypoproteinemia	2	2	2			2		2	(9)
	Lean meat	Plasma injections	3	2		1	1	1			(14)
	Total		33	21	10	7	4	16	3	13	
	Percentage with lesions			(64)	(30)	(21)	(12)	(48)	(9)	(39)	
Mercuric chloride	Kennel	None	9	7		2	1	2	2	1	(3)
		Citrate injections	2	2	1			1	1	1	(4)
	"Standard"	None (6 wks. of diet)	3	3	1			1	1	2	(10)
		Plasma injections	3	3	2	1	1	3	1	2	(11)
	Lean meat	Plasma injections	1								(15)
	Total		18	15	4	3	2	7	5	6	
	Percentage with lesions			(83)	(22)	(17)	(11)	(39)	(28)	(33)	
Bilateral nephrectomy	"Standard"	None (20 wks. of diet)	3	3	1	1		2	1	2	(12)
		Plasma injections	3	3	3			3	2	3	(13)
	Total		6	6	4	1		5	3	5	
	Percentage with lesions			(100)	(67)	(17)		(83)	(50)	(83)	
	Total for all dogs		57	42	18	11	6	28	11	24	
	Percentage with lesions			(74)	(32)	(19)	(11)	(49)	(19)	(42)	

* These subgroup numbers correspond to those in Table I.

have varied from a few millimeters in diameter to massive lesions involving practically the entire fundic portion. The gastric lesions were confined to the acid-secreting portion. In the most advanced case (dog 40–63, subgroup 11) there was a heavy hemorrhagic fibrinous exudate which formed a membrane that covered the fundus. This measured 8 mm. in its thickest portion. Histologically the early lesions were characterized by hemorrhage in the mucosal stroma and edema in the submucosa. In both of these locations there were slight deposits of fibrin and early infiltration with polymorphonuclear neutrophils. As the lesions became more advanced, fibrin and polymorphonuclears with varying degrees of necrosis occupied a more conspicuous part of the picture, but even in the most advanced lesions hemorrhage and edema were the predominant features. In 2 of the dogs with gastric lesions there were acute necrotizing lesions in the arterioles in the submucosa (Fig. 6), but there was no obvious cause-and-effect relationship between these two concurrent lesions.

In the small intestine the lesions consisted principally of scattered clusters of small hemorrhages. A typical cluster contained ten to fifteen more or less rounded hemorrhages measuring about 3 mm. in diameter, the whole confined to a segment of the bowel measuring about 6 cm. in length. These clusters of petechial hemorrhages occurred in every portion of the small intestine but were found most frequently in the duodenum and upper jejunum. When there was parasitic infestation (*e.g.*, hookworms) in this portion of the bowel the lesions bore no relationship to the parasites; and other dogs with heavier parasitic infestation had no intestinal lesions in the sense used in this paper. Histologically these lesions were similar to those in the stomach but were not as massive. One dog had an intussusception of its ileum.

In the colon the lesions tended to be most prominent in the cecum and in the ascending portion. Here hemorrhagic streaks along the crests of the longitudinal folds characterized the picture. In two of the dogs, patchy areas of shallow ulceration covered by a delicate membranous exudate were present about these hemorrhagic streaks. Microscopically the lesions were similar in character to those in the stomach and small intestine.

In all three segments of the gastrointestinal tract many more lesions were diagnosed grossly than were confirmed histologically. Only those confirmed by microscopical study have been included in this series. It is possible that a single microscopical section failed to show the lesion and that the incidence of lesions listed in Table II is too low; but in those cases with suspected gross lesions, microscopical study

revealed sufficient congestion to account for the gross appearance. Had these dogs lived longer, the congestion might have progressed to hemorrhage, exudation and necrosis. This same argument, however, could be advanced for every capillary in the body, hence it was thought best to include only those lesions confirmed by histological study.

Clinically these gastrointestinal lesions were usually associated with bloody diarrhea during the last day or two of life. This fact and the gross and histological appearance of the lesions, specifically the absence of sloughing and deep ulceration, make it probable that these lesions occurred within 3 days or less prior to death. Analysis of the data in Table II indicates that these lesions were not necessarily related to diet or to the method by which renal insufficiency was produced. The highest percentage of incidence was in the group subjected to bilateral nephrectomy. This excludes heavy metal as the causative agent, but the number of dogs is too small to attach any significance to the increased incidence of lesions in this group. As in the oral cavity, the lesions apparently started beneath intact epithelium and only after superficial ulceration did intestinal organisms add their enzymatic action to the inflammatory response. The factor responsible for these gastrointestinal lesions remains obscure just as it does for those in human cases of mercurial gastroenteritis.

Pancreatic Fat Necrosis

The third lesion that occurred with some degree of regularity in this series of dogs subjected to renal insufficiency is pancreatic fat necrosis. In no instance were these grayish white areas of necrosis particularly conspicuous, and it is questionable whether they played any significant part in the summation of chemical reactions leading to death. It seems more likely that they represent another anatomical evidence of a more fundamental disturbance associated with renal insufficiency. They were usually located in the peripancreatic fat; the single, most frequent site was the head of the dorsal pancreas near the duodenum. Occasionally they have been found in the periadrenal fat and in the areolar tissue about the ovaries, and in one instance in the mesentery. No lesions as large as 1 cm. in maximum diameter were encountered. Most of the lesions measured 2 to 3 mm. in maximum diameter and consisted of a small gray or grayish white dot. Sometimes the borders of these areas were reddish, but in no instance was any definite gross hemorrhage observed in association with these lesions. Microscopically these lesions were typical pancreatic fat necroses, usually with massive polymorphonuclear leukocytic reaction in, and especially about, the central area of fat

necrosis. The slurred bluish gray material (seen in sections stained with hematoxylin and eosin) that filled the space previously occupied by the fat cells was conspicuous in the central portion. In a few cases, sheaves of fatty acid crystals have been seen in this material. One of the lesions showed evidences of healing.

There were no clinical symptoms referable to these pancreatic fat necroses. They were not related to diet or to the method by which renal insufficiency was produced. Again their more frequent occurrence in the dogs subjected to bilateral nephrectomy excludes heavy metal from any etiological rôle. The factor responsible for these lesions is also obscure.

Other Lesions

In addition to the three lesions described, the following lesions have occurred in one or more of the dogs detailed in Table I:

(a) Hemorrhages in epicardium, usually most marked along the atrioventricular sulcus.
(b) Hemorrhages in endocardium. These have been most conspicuous in the left ventricle at the tips of the papillary muscles and in the septum below the aortic valve. Rarely has one of these hemorrhages been associated with necrosis of the myocardium.
(c) Acute mitral valvulitis.
(d) Edema of the tricuspid valve.
(e) Hemorrhages in thymus.
(f) Atrophy of lymphoid tissue.

These lesions seem to be related to the experimental procedures but their incidence has not been sufficiently great to warrant statistical analysis. Incidental findings, such as parasitic infections and infestations and terminal pneumonia, have not been included.

Discussion

The lesions described in this paper (stomatitis, gastroenteritis and pancreatic fat necrosis) appear to be independent of diet. This contrasts sharply with the arterial lesions (described in the preceding paper [1]) in which diet seems to play a definite rôle. The lesions in the gastrointestinal tract, also some of the "other lesions" listed, are similar to those described by Winternitz and his co-workers.[7, 8] These are discussed in the preceding paper.[1] No mention was made by these workers of mouth lesions or lesions in the peripancreatic fat.

Renal insufficiency is the only factor common to all of the lesions (including arterial lesions). The method by which renal insufficiency

is produced is unimportant. The fact that all of the lesions occurred as frequently, or more frequently, in dogs subjected to bilateral nephrectomy eliminates heavy metal *per se* from a pathogenic rôle. If these findings are confirmed for man, and I know of no contradictory evidence, "mercurial" stomatitis, gastritis and colitis are probably due not to mercury as such but to the renal insufficiency produced by the heavy metal.

We have no evidence as to the nature of the factor associated with renal insufficiency that is responsible for these lesions, *i.e.*, whether there is a deficiency of a necessary factor, a "toxic" factor, or an "imbalance."

The rate of development of renal insufficiency seems to be important and probably explains why all human cases of "uremia" do not develop these lesions. Whatever the responsible factor happens to be, it must occur at such speed that local breakdown products (degenerated collagen?) accumulate in sufficient concentration to disturb local equilibria and lead to hemorrhage and necrosis. Do the sites of predilection for the lesions—three in the mouth (upper lips, cheeks and under surface of the distal half of the tongue near the lateral margins), the stomach, the upper portion of the small intestine and the first portion of the colon, three in the arterial system (pulmonary artery, endocardium of the left auricle and ascending portion of the arch of the aorta including the mouth of the innominate artery) and the peripancreatic fat—have any anatomical or physiological peculiarities that would help explain these lesions? This question cannot be answered at this time, but the predictable frequency with which these lesions occur at definite anatomical sites, especially the three localized areas in the mouth and the three areas in the arterial system, following a general metabolic disturbance such as renal insufficiency (or renal insufficiency plus diet in the case of the arterial lesions) suggests that competitive affinities for a necessary factor or increased susceptibilities to a toxic factor may be in operation. I am inclined to favor the former view, namely, competitive affinities for a necessary factor, possibly because of the influence of diet on the incidence of arterial lesions, but lack of proof must be admitted.

Another feature of these lesions is noteworthy but I hesitate to do more than mention it for fear of getting sidetracked in a philosophical discussion. These lesions fill all the criteria for acute inflammation, yet as far as I can determine they are due only to alterations in kidney function (and diet in the case of the arterial lesions). There seems to be little or no doubt that the lesions start beneath intact epithelium in the case of the mouth and gastrointestinal lesions, be-

neath intact mesothelium in the case of the pancreatic fat necrosis and beneath intact endothelium in the case of the arterial lesions. Only when necrosis with sloughing and ulceration has opened a portal of entry to mouth and intestinal organisms do bacteria play a part in the inflammatory reaction. In the arterial lesions and the areas of pancreatic fat necrosis all attempts to demonstrate organisms by cultural methods or by bacterial stains have been negative. All of the control data [2] and the fact that the development of the lesions has always corresponded to the interval between the production of renal insufficiency and death militate against an infectious agent. The evidence that the lesions are due to an "internal irritant" seems to be valid. Other probable examples of the same phenomenon (inflammatory lesions due to an "internal irritant") could be cited, both in animal experiments [9-11] and in human pathology (periarteritis nodosa, muscular dystrophy), but it is not the purpose of this paper to discuss the mechanisms of inflammation.

Summary

1. In a group of 57 dogs subjected to renal insufficiency by one of three methods (uranium nitrate in 33, mercuric chloride in 18, and bilateral nephrectomy in 6) the following lesions, in addition to the arterial lesions described in the preceding paper,[1] were found in the stated percentage of cases:

(a) Acute necrotizing stomatitis in 42 (74 per cent)

(b) Acute hemorrhagic gastroenteritis in 28 (49 per cent)

(c) Acute pancreatic fat necrosis in 11 (19 per cent)

2. The occurrence of all three lesions in dogs subjected to bilateral nephrectomy seems to eliminate heavy metal *per se* from any pathogenic rôle in the development of these lesions.

3. The "irritant" responsible for the inflammatory reaction in all of these lesions appears to be an "internal" one related to the disturbed metabolism associated with renal insufficiency.

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DESCRIPTION OF PLATES

PLATE 123

Acute necrotizing stomatitis. All sections were stained with hematoxylin and eosin.

FIG. 1. Dog 42–5. Typical upper lip lesions.

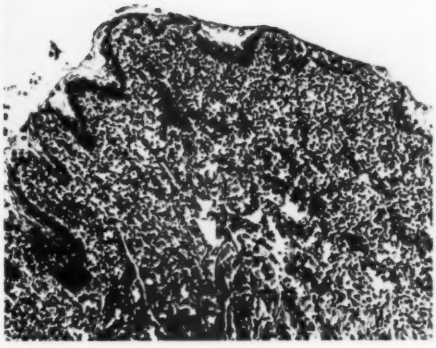
FIG. 2. Dog 40–84. Upper lip. Intense leukocytic reaction beneath remnants of squamous epithelium. × 100.

FIG. 3. Dog 41–93. Tongue. Extensive necrosis and leukocytic infiltration without excavating ulceration. × 84.

FIG. 4. Dog 39–36. Tongue. Congestion, edema, hemorrhage, polynuclear infiltration and necrosis with relatively slight changes in the surface epithelium. × 135.

FIG. 5. Dog 39–49. Tongue. Extensive leukocytic infiltration and necrosis with remnants of the surface epithelium still recognizable. × 140.

1



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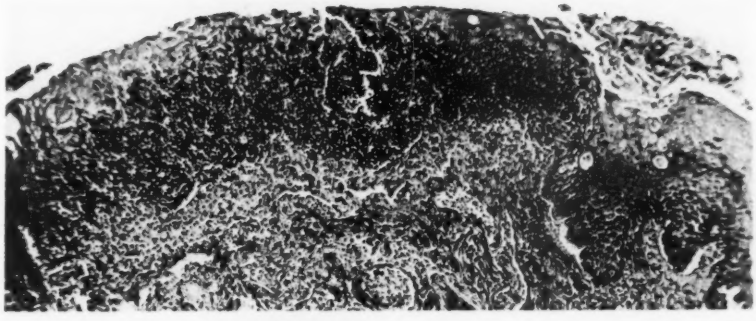

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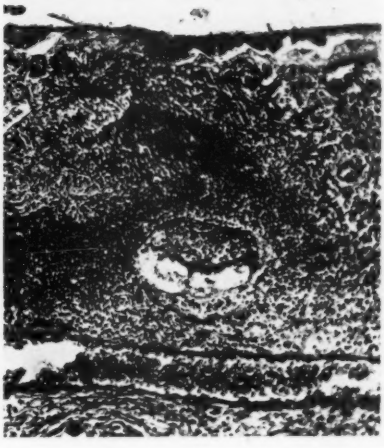


5

Holman Experimental Necrotizing Arteritis. VI

PLATE 124

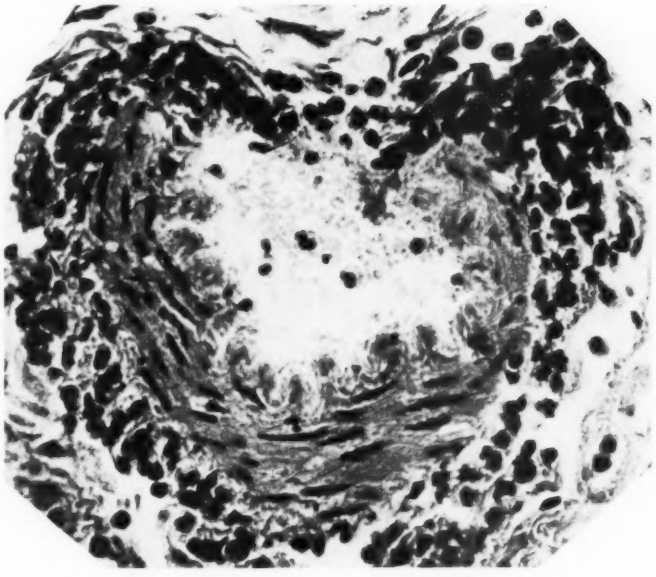
All sections were stained with hematoxylin and eosin.

FIG. 6. Dog 42–8. Acute periarteritis of arteriole in submucosa of stomach. × 580.

FIG. 7. Dog 41–94. Hemorrhage and early leukocytic infiltration in mucosal stroma of ileum. × 100.

FIG. 8. Dog 40–63. Acute pancreatic fat necrosis of peripancreatic fat. × 100.

6



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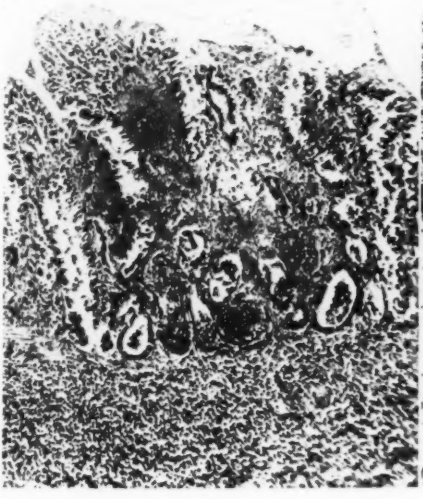

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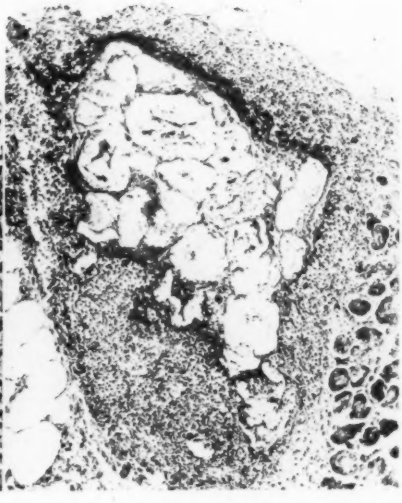

Holman Experimental Necrotizing Arteritis. VI

BACTERIOLOGICAL OBSERVATIONS ON EXPERIMENTAL BRUCELLOSIS IN DOGS AND SWINE*

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From the time of the earliest investigation of brucellosis it has been recognized that the host-parasite relationship in this disease presents a peculiar and puzzling problem. As the human disease has become better known the problems have multiplied, and it has become desirable to attempt a solution of them through investigations of the disease in the experimental animal. Furthermore, it has been suggested by recently reported studies [1-3] that brucella may be recovered from a considerable proportion of the cases of Hodgkin's disease, and, at the same time, it has been observed that there are striking similarities between human brucellosis and Hodgkin's disease.[1, 4] The latter observations emphasize more than ever the need for more precise information concerning the relation between brucella and its hosts since only through such information can one expect to form an intelligent judgment of the significance of the data in those instances of a co-existence of brucellosis and Hodgkin's disease. In the latter connection, and also in studying the usual form of human brucellosis, one is faced with certain serious difficulties; the more immediately important of these may be stated briefly as follows: (1) As yet there appears to be no absolutely reliable method for the isolation of brucella from the tissues. (2) In cases of brucellosis proved by the recovery of brucella from the blood, the bacteremia appears to be intermittent, the time between its repeated occurrence being extremely variable; this condition is impossible to understand until the reservoir of the organisms responsible for these recurrences of the bacteremia is known with certainty. (3) The precise relation of the cells of the natural host to the organisms that may be obtained from the tissues by culture is not fully understood. (4) The factors which determine the prolonged course and the recurrence of attacks in chronic brucellosis have not been determined. (5) The influence of the host tissues upon the pathogenicity of organisms which remain in the tissues for long periods is only approximately known. (6) The specific mechanisms through which brucella provokes the different defense mechanisms of the host are little understood.

* This work was supported by a grant from the John and Mary R. Markle Foundation, and Duke University Research Council.

Received for publication, February 11, 1943.

During the past 2 years we have carried out a series of studies of experimentally induced brucellosis in a variety of animals in an attempt to solve some of these problems. A preliminary report of the general pathological findings in these studies as they relate to the dog and the hog may be found in the Proceedings of the American Association of Pathologists and Bacteriologists.[5] The report which is to follow covers the bacteriological and immunological observations that were made on this same group of animals. In our experiments on the hog, a natural host, and the dog, an unusual host, our chief object was to learn, first, how long the organisms remain in the tissue when they are no longer demonstrable in the peripheral blood, and, second, under such circumstances, in what tissues they may be found most frequently.

Instances of proved spontaneous brucellosis in the dog are few. Kennedy and Eyre[6] obtained four positive agglutination tests for *Brucella melitensis* in 162 dogs tested in Malta. The bacterium was isolated from the tissues of one of these dogs at autopsy. Plantz and Huddleson[7] reported the isolation of *Brucella suis* in pure culture from a testicular abscess in a 3½-year-old dog with a blood serum agglutinating titer of 1:500 by rapid test. Van der Hoeden[8] isolated brucella from the blood of a dog with high agglutinating titer by inoculating a guinea-pig with the blood. Two other dogs with high blood serum titers yielded negative blood cultures. The organism could not be recovered from the liver or the kidney of any of the 3 animals either by direct culture or by guinea-pig inoculation. Reports of the occurrence in dogs of specific agglutinins for brucella have been made by various investigators. This is reviewed by van der Hoeden,[8] Caliri[9] and Feldman, Mann and Olson.[10] In van der Hoeden's[8] review are reported the results of his own experiments in which 11 dogs were infected *per os* with various strains of brucella. Agglutinating and complement-fixing antibodies were regularly found in these animals from 6 days to 3 months following introduction of the organisms. At autopsy, brucella was recovered from 7 animals, the spleen, liver, bone marrow and mesenteric lymph nodes yielding the higher percentages of positive cultures (54.5 to 57.1 per cent). The organism was recovered less frequently from the blood and rarely from the kidneys, bile, or testis. One dog receiving heat-killed brucella *per os* failed to develop demonstrable serum antibodies during the 35 days it was observed. In a later investigation, van der Hoeden[11] reported a group of experiments in dogs in which infection had been produced *per os*, percutaneously by way of the conjunctiva, and by contact with dogs previously infected *per os*. These animals were killed from 1 to

135 days following infection. Brucella was recovered more frequently from the spleen, liver, bone marrow and regional lymph nodes (47.6 to 65.2 per cent), less frequently from the blood (22 per cent) and rarely from kidneys and testis. Van der Hoeden called attention to the most frequent involvement of parts of the reticulo-endothelial system. Feldman, Bollman and Olson [12] recovered brucella at autopsy from 2 of 5 dogs infected intravenously. One of these animals had positive blood and urine cultures when sacrificed on the 39th day. The other did not yield positive cultures during life; at autopsy, 185 days after the inoculation, the organism was recovered from a mesenteric lymph node. Of 6 dogs infected *per os*, 3 yielded positive blood cultures on the 14th day, but no positive cultures were obtained at autopsy. On the basis of these observations Feldman, Bollman and Olson expressed the opinion that spontaneous brucellosis in dogs is rare, since this animal apparently possesses a highly efficient mechanism for protection against *Brucella abortus*.

Both spontaneous and experimental infections with brucella in swine have received considerable attention in recent years. Good and Smith [13] isolated brucella from the fetuses of an aborting cow and inoculated this organism into two pregnant sows intravenously and *per os*. The sows aborted 17 and 19 days, respectively, after infection, and from the fetuses of each brucella was recovered. Weeter [14] reviewed the literature dealing with the early investigations of spontaneous infection of swine and contributed further observations on spontaneous and experimental infection of swine with brucella. In Weeter's observations on spontaneous infection, the organism was isolated three times from 259 nongravid uteri of swine and once from 289 gravid uteri. His attempts to infect adult swine *per os* resulted in the production of agglutinating antibodies in the serum of the animals, but the initial infection and subsequently ingested organisms soon were eliminated completely as indicated by failure to recover the organisms. Young animals showed a high resistance to oral infection by Weeter's cultures. Cotton and Buck [15] found that boars and pregnant sows could be infected regularly with *Brucella abortus* (*suis*) through the conjunctiva. The organism was recovered by inoculating guinea-pigs with the blood from 12 of 13 animals infected in this way. Five of the animals yielded positive blood cultures as long as 6 to 7 weeks following infection; positive blood cultures on the other 7 animals were obtained not longer than 2 to 4 weeks following infection. Brucella was recovered from the liver of 1 sow killed 102 days after infection. The organism was not recovered at autopsy from another sow killed after 102 days, nor from a sow that

died 48 days following infection. Feldman and Olson [16] isolated brucella from 11 of 24 swine with spondylitis. These animals apparently were symptomless and showed no lesions in other parts of the body than the spine. Feldman and Olson [17] also isolated brucella by guinea-pig inoculation from 2 of 102 apparently normal swine. The organisms were recovered from the lymph nodes on the head and in the anterior cervical region; they were also obtained from an abscess of the spermatic cord of one of the animals and from the spleen of the other animal. Gilman, Milks and Birch [18] passed bovine strains of brucella through a series of hogs in experiments which demonstrated the failure of such animal passage to cause bovine strains to assume the characteristics of the porcine type. In the course of these investigations, large doses of organisms were administered intravenously to 18 sows. Brucella was not recovered by guinea-pig inoculation from 2 of the animals, killed at the end of 103 and 59 days respectively. The remaining 16 sows were killed from 21 to 57 days following infection. Brucella was recovered from the lymph nodes of all 16 animals, from the spleen in 4, from the liver in 1, from the ovaries and uterine wall in 4, and from the mammary gland in 4; in no instance was the organism recovered from the blood. Feldman and Olson [19] introduced *Brucella suis* into swine by way of the conjunctiva, intravenously, orally and subcutaneously, and sacrificed the animals 319 days following infection. They found no lesions in these animals and failed to recover the organism by guinea-pig inoculation. From this experiment these workers concluded that swine apparently possess considerable natural resistance to experimental infection with one strain of brucella of porcine origin.

The experience of previous investigators of brucellosis in the dog and the hog thus indicates (1) that both the dog and the hog appear to possess considerable natural resistance to experimental infection with brucella, (2) that positive agglutination tests are not necessarily indicative of infection that can be demonstrated by recovery of brucella from the animal, and (3) that when the organisms are recovered, they are most frequently found in "integral parts of the reticulo-endothelial system" (van der Hoeden [11]).

Materials and Methods

In the present study, our procedures and observations were as follows:

Two strains of *Brucella suis* were selected for inoculation into the test animals: Strain A was isolated from the spleen of a naturally infected hog and was highly virulent for guinea-pigs, regularly producing

gross lesions and often death within 3 to 4 weeks after intraperitoneal inoculation. Strain B was obtained originally at autopsy from a case of Hodgkin's disease; at the time of these experiments, its virulence for guinea-pigs was slight.

Repeated inoculations of the test animals were made at intervals of from 7 to 21 days. In each instance, a freshly prepared bacterial saline suspension from a 48-hour agar slant culture standardized by means of the photronreflectometer was used.

Blood samples were cultured in Bacto-tryptose* broth with subculture to Bacto-tryptose sheep blood agar slants. Tissues obtained at autopsy were ground with sterile alundum and physiological saline solution to produce suspensions; small portions of these were streaked on Bacto-tryptose sheep blood agar plates, the remainder being added to poured plates of the same medium. The tissues selected for culture are noted in Tables I and II. In every instance, lymph nodes from at least two different sites were studied.

EXPERIMENTS

Dogs

Six male and 3 female adult mongrels were selected. Each appeared to be in good condition and weighed about 10 Kg. Preliminary brucella agglutination tests were negative, and brucella opsonocytophagic readings were within normal limits. Each animal was given an inoculum of 10 billion organisms at each injection. Four animals received repeated doses of *Br. suis* (strain B) intravenously; 1 animal, *Br. suis* (strain A) intravenously; 3 animals, *Br. suis* (strain B) intraperitoneally; and 1 animal, *Br. suis* (strain A) intraperitoneally. The dogs receiving *Br. suis* (strain B) were inoculated every 7 days for 105 days (except when it seemed doubtful that the animal would survive an inoculation), and every 21 days thereafter. The 2 dogs receiving *Br. suis* (strain A) were inoculated at 1, 14 and 34 days. The duration of the experiment for each animal is recorded in Table I. All dogs were bled from the jugular vein at frequent intervals for blood culture, brucella agglutination and opsonocytophagic tests.

The bacteriological findings are summarized in Table I and, together with the immunological data, are discussed later.

Swine

Six male and 2 female hogs, 6 weeks of age, were selected from a single litter. Preliminary brucella agglutination tests and blood cultures of both test animals and parent sow were negative. Preliminary

* Product of Difco Laboratories, Inc., Detroit, Mich.

TABLE I

Experimental Data for Dogs, Including Results of Blood and Tissue Cultures

Dog no.	1	4	5	2	10	9	3	6	7
Brucella suis strain received	B	B	B	B	A	A	B	B	B
Inoculations*	21/i.v.	28/i.v.	4/i.v.	35/i.v.	3/i.v.	3/i.p.	39/i.p.	39/i.p.	33/i.p.
Blood cultures†	12/27	23/34	3/5	18/42	3/5	1/5	4/41	0/43	8/34
Duration (in days) of experiment	198	261	38	487	216	186	461	454	398
Fate of animal	Died	Died	Died	Killed	Killed	Killed	Killed	Killed	Killed
Days between last inoculation and death	14	7	8	225	182	152	143	136	132
Days since last positive blood culture	7	7	1	233	101	152	270	None	231
Autopsy cultures:‡									
Spleen	+	+	+	−	−	−	−	−	−
Liver	+	+	o	−	−	−	−	−	−
Kidney	+	+	o	+	−	−	−	−	−
Lymph nodes	+	o	+	+	+	+	−	−	−
Testis	−	+	o	−	o	−	o	−	o
Lung	−	−	o	o	o	o	o	o	−

* Numerator refers to the number of inoculations given, denominator to the route of inoculation. i.v. = intravenous; i.p. = intraperitoneal.

† Numerator refers to the number of positive blood cultures obtained, denominator to the total number of blood cultures done.

‡ o = not cultured; + = positive for brucella; — = negative for brucella.

opsonocytophagic indices of the test group ranged from 2.5 to 8.5. During the experiments all animals were bled at frequent intervals from the tail, the femoral vein, or the jugular vein for blood cultures, brucella agglutination and opsonocytophagic tests.

At intervals of from 4 to 8 days for 98 days, hogs 1 to 6 were inoculated intraperitoneally with *Br. suis* (strain B) in doses started at 70 million and increased by small amounts to 30 billion organisms. Beginning with the 15th week of the experiment, intravenous inoculations of 30 billion organisms were administered to hogs 1 to 5 every 7 to 14 days for the next 112 days, every 21 to 35 days thereafter. By means of a rubber catheter fitted with a syringe, hog 7 was given orally 7 doses of 30 billion *Br. suis* (strain A) organisms at 1, 6, 27, 42, 48, 70 and 111 days. Hog 8 received the same doses intravenously with omission of the 42nd day.

The bacteriological findings are summarized in Table II.

Discussion

Nine dogs were subjected to large and repeated inoculations of *Br. suis*, 5 animals receiving the organisms intravenously and 4 intraperitoneally. The 5 dogs receiving *Br. suis* intravenously showed a high percentage of positive blood cultures when tested from 6 to 14 days following an inoculation; blood samples taken after 14 days yielded no growth, except in one instance (dog 10). Three of the 4 dogs receiving *Br. suis* intraperitoneally occasionally had positive blood cultures, 20 days being the longest observed period between inoculation of organisms and recovery of brucella from the blood. The greater incidence of positive blood cultures in the intravenous group is striking (Table 1): 42.9 per cent (18 of 42) to 67.6 per cent (23 of 34) positive, as contrasted to 0 to 23.5 per cent (8 of 34) in the intraperitoneal group.

No such difference was observed in agglutination and opsonocytophagic tests. In all of the dogs, regardless of route of inoculation or strain of *Br. suis* employed, the agglutination titer for *Brucella abortus* (456) rose to 1:10,240 or higher within 7 to 14 days following the first inoculation and remained at that level until termination of the experiment. The opsonocytophagic indices also rose within 7 to 14 days following the first inoculation and tended to remain high throughout the experiment. Wide individual variations were observed from week to week, but these could not be correlated in any way with the course of the infection. Two control animals, each of which was given three intravenous inoculations of 10 billion heat-killed *Brucella suis* (strain B) organisms at 1, 14 and 35 days, showed a similar

TABLE II

Experimental Data for Hogs, Including Results of Blood and Tissue Cultures

Hog no.	2	3	5‡	1	4	8	6	7
Brucella suis strain received	B	B	B	B	B	A	B	A
Inoculations* (i.p. or p.o.)	15/i.p.	14/i.p.	13/i.p.	15/i.p.	14/i.p.	—	14/i.p.	7/p.o
Blood cultures in above period†	0/7	0/7	0/6	0/7	0/7	—	0/5	0/6
Subsequent inoculations (i.v.)*	17/i.v.	17/i.v.	18/i.v.	14/i.v.	18/i.v.	6/i.v.	—	—
Subsequent blood cultures†	9/17	7/17	9/17	12/14	7/17	2/6	—	—
Duration (in days) of experiment	436	411	447	245	424	242	98	240
Fate of animal	Killed	Killed	Killed	Killed	Killed	Killed	Died	Killed
Days between last inoculation and death	117	92	128	10	105	131	7	129
Days since last positive blood culture	180	133	169	0	168	208	None	None
Autopsy cultures:‡								
Spleen	—	—	—	+	—	—	—	—
Liver	—	—	—	+	—	—	—	—
Kidney	—	—	—	—	—	—	0	—
Lymph nodes	—	—	—	+	+	+	—	—
Testis	—	—	—	—	0	+	0	0
Lung	0•	0	0	+	0	0	0	0

* Numerator refers to the number of inoculations given, denominator to the route of inoculation. i.p. = intraperitoneal; p.o. = *per os;* i.v. = intravenous.

† Numerator refers to the number of positive blood cultures obtained, denominator to the total number of blood cultures done.

‡ 0 = not cultured; + = positive for brucella; — = negative.

§ Spleen culture positive for brucella at operation on 87th day of experiment.

rise in brucella agglutination titer and opsonocytophagic index during the 157-day period in which they were followed.

At autopsy brucella was recovered from all of the dogs in the intravenous group and from 1 dog* in the intraperitoneal group. Our chief purpose was to determine, if possible, first, how long the organisms remain in the tissues when they are no longer demonstrable in the peripheral blood, and, second, under such circumstances, in what tissues they are most frequently found. Three dogs (nos. 1, 4 and 5) of the intravenous group died on the 198th, 261st and 38th day respectively. In all three instances, the last inoculation and the last positive blood culture occurred too recently to make the positive autopsy culture significant. Three dogs (nos. 3, 6 and 7) of the intraperitoneal group, killed on the 461st, 454th and 398th day respectively, yielded no positive cultures at autopsy. The remaining 3 dogs, 2 (nos. 2 and 10) of the intravenous group and 1 (no. 9) of the intraperitoneal group, were killed on the 487th, 216th and 186th day respectively, and the organism was recovered from each dog at autopsy. In these three instances, a significantly long period of time had elapsed since the last inoculation of organisms or the last positive blood culture (225, 101 and 152 days respectively). So, in these 3 dogs, the tissues from which the organisms were isolated at autopsy are of interest. Spleen, liver, kidney and lymph nodes were cultured in each instance, as well as testis from 2 of the 3 animals. Brucella was recovered from lymph nodes of all 3 dogs. The only other positive culture was obtained from the kidney of dog 2 (Table 1).

Essentially the same experiment was performed on hogs, using repeated inoculations of *Br. suis*. Of the 8 hogs employed, 1 was inoculated orally, 1 intraperitoneally, 5 intraperitoneally initially and then intravenously, and 1 intravenously. Blood cultures on the intraperitoneal and intraperitoneal-intravenous groups were uniformly negative during the 98-day period of intraperitoneal inoculations. During this time, however, on the 87th day of the experiment, 8 days after the 13th intraperitoneal inoculation of organisms, a culture of the spleen obtained at operation from 1 animal (hog 5) was positive for *Br. suis*; cultures of the liver, the peritoneal cavity, a mesenteric lymph node and the bile, made at the same time from this animal, were negative.

* Dog 9 was the one animal of the intraperitoneal group of 4 to be given *Br. suis* (strain A). This was the only evidence encountered to indicate greater virulence of *Br. suis* (strain A) over *Br. suis* (strain B) for either the dog or the hog, despite the marked difference known to exist between the two strains when inoculated into guinea-pigs. The lack of further evidence was particularly surprising in the experiments on the hog, inasmuch as strain A was isolated from a naturally infected hog and might have been expected to be highly virulent when re-introduced into the natural host.

Blood cultures from the orally inoculated animal (hog 7) were uniformly negative. In the group of 6 hogs subsequently receiving intravenous inoculations, the percentage of positive blood cultures was high (Table II): 33.3 per cent (2 of 6) to 85.7 per cent (12 of 14) as contrasted to none obtained during intraperitoneal or oral inoculation. As in the dogs, all cultures made more than 21 days following an inoculation were negative.

Within 40 to 55 days following the first inoculation, all 8 of the animals except hog 7 possessed agglutination titers for *Brucella abortus* (456) of 1:10,240 or higher. These titers were maintained throughout the experiment with two exceptions: The final readings done on hogs 3 and 5 showed titers of 1:5,120 and 1:2,560 respectively, and hog 7 receiving *Brucella suis* (strain A) orally possessed titers ranging irregularly from 1:320 to 1:5,120 from the 7th day until termination of the experiment. Opsonocytophagic indices of all of the animals were variable and not significantly elevated at any time.

The duration of the experiment for each animal is recorded in Table II. No positive cultures were obtained at autopsy from the 2 animals (hogs 6 and 7) receiving inoculations intraperitoneally and orally respectively. Positive autopsy cultures were obtained from 3 of the 6 intravenously inoculated animals.

As in the dogs, we had few animals which could be used to determine the presence of organisms in tissues when they were no longer demonstrable in the peripheral blood. Hog 1 of the intraperitoneal-intravenous group, killed on the 245th day, had a positive blood culture when sacrificed. Two animals (nos. 4 and 8) yielded positive autopsy cultures when killed on the 424th and 242nd day respectively. Both had received intravenous inoculations, their last inoculations or positive blood cultures occurring 105 and 131 days respectively before death. Spleen, liver, kidney and lymph nodes were cultured in each instance, as well as a testis of hog 8. Both animals (nos. 4 and 8) yielded positive lymph node cultures. In addition, brucella was recovered from the testis of hog 8.

Conclusions

1. Brucella usually disappears from the blood stream of both dog and hog within 1 to 3 weeks after an inoculation of organisms, but it can be recovered from the tissues in some instances from 3 to 7 months later.

2. In the absence of a positive blood culture and of all evidences of clinical infection at the time of autopsy, from inoculated dogs and hogs brucella was recovered most frequently from lymph nodes.

In an equal number of instances, the organism was not recovered from any site.

3. When *Brucella suis* is introduced intravenously into dogs and hogs, infection is frequently established, as shown by repeated recovery of the organism from the blood during life or from the tissues at autopsy; but animals so infected may present no clinical evidence of disease. It is extremely difficult to establish infection (demonstrable by recovery of brucella from the animal) by intraperitoneal inoculation. Infection of one hog by the oral route could not be confirmed by recovery of the organism.

4. The brucella agglutination titers of both dog and hog tend to rise early in the course of experimental inoculation with brucella and to remain high. Furthermore, the agglutination titer apparently is not materially influenced by the route of inoculation or the strain of *Br. suis* employed for inoculation; nor was any difference observed in this connection when heat-killed organisms were used instead of live organisms.

5. The brucella opsonocytophagic indices of both dog and hog were variable and could not be correlated in any way with the course of the experimental infection.

6. No striking difference in virulence between the two strains of *Br. suis* employed for the dog and the hog was observed despite the fact that for the guinea-pig the animal strain A was highly virulent and the human strain B only slightly so.

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THE EFFECT OF THE LEUKOCYTOSIS-PROMOTING FACTOR ON THE GROWTH OF CELLS IN THE BONE MARROW*

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Earlier studies made by me and my associates [1-3] have demonstrated the existence of a leukocytosis-promoting factor in numerous types of inflammatory exudates derived from various animal forms, including man. The presence of this factor offers a reasonable explanation for the mechanism of leukocytosis accompanying numerous inflammatory and infectious processes, for upon introduction of the exudative material into the blood stream of dogs or rabbits there follows, within a few hours, a discharge of immature leukocytes into the circulation.[1] This part of the study has recently been confirmed in rabbits by Reifenstein, Ferguson and Weiskotten.[4] The prompt effect on the leukocyte level cannot be elicited by normal blood serum, bacterial suspension, nucleic acid, adenosine, or histamine.[1]

Heating the exudate to a temperature of about 60°C. inactivates it.[1] Furthermore, dialysis indicates that the leukocytosis-promoting factor is evidently nondiffusible.[2] These findings have suggested the protein nature of the factor. Thereupon, fractionation with ammonium sulfate at various concentrations has been undertaken.[5] The active factor has been isolated as a pseudoglobulin.[5] In the earlier technic of extraction the euglobulin fraction of exudates was often retained with the pseudoglobulin. Furthermore, the injection of such fractions (as well as the untreated exudate) into the blood stream of dogs was frequently followed by a transient leukopenia which was subsequently replaced by a leukocytosis. The initial drop in the level of circulating leukocytes has recently been found to be referable to the presence of an injury factor in exudates, capable *per se* of explaining the fundamental pattern of cellular damage in inflammation.[6] This factor is found to be associated with the euglobulin fraction of exudates. To it the term "necrosin" has been assigned.[6] Besides inducing severe tissue injury accompanied by lymphatic blockade, necrosin, introduced into the circulation, is followed by a marked leukopenia. The polymorphonuclear leukocytes seem to be obliterated from the circulation leaving a differential leukocyte count characterized by a relative lympho-

* Aided by grants from the Jane Coffin Childs Memorial Fund for Medical Research, the International Cancer Research Foundation, and the Daland Fund of the American Philosophical Society, and from a grant under a contract from the Office of Scientific Research and Development.

This article is paper XXV of a series entitled "Studies on Inflammation."

Received for publication, January 15, 1943.

cytosis. It is my opinion that the liberation of sufficient necrosin at the site of injury may offer a reasonable explanation for the leukopenia accompanying some infectious processes. These studies have been reported *in extenso* in a separate communication.[6a] At any rate it is clear that the initial leukopenia associated with the introduction of the leukocytosis-promoting factor can be readily disposed of by preliminary removal of the euglobulin fraction from exudates.

The present scheme adopted for the extraction of the leukocytosis-promoting factor can be briefly summarized as follows:

Exudate or cell-free exudate
↓
$(NH_4)_2SO_4$ at ⅓ saturation and centrifuge
↓ → precipitate (euglobulin or "necrosin")
Supernatant
↓
$(NH_4)_2SO_4$ at ⅓ saturation, or even ½ saturation
↓
Dialyze precipitated mixture for 16 to 24 hours against tap H_2O
↓
Reprecipitate residual nondiffusible material at ½ saturation $(NH_4)_2SO_4$
↓
Centrifuge
↓ → supernatant
Pseudoglobulin precipitate taken up in distilled H_2O
↓
Dialyze against tap H_2O until free of $SO_4^{=}$
↓
Residual pseudoglobulin (leukocytosis-promoting factor)

The leukocytosis-promoting factor can be readily desiccated in a vacuum oven at about 35°C. or it can be dried by freezing in a Flosdorf-Mudd apparatus.[7] The active material appears in the purified fractions as a whitish, rather brittle powder but which, with some adhering impurity, may have a trace of light brown color. The desiccated material is stable. When kept in a refrigerator for about 6 months it has been found unimpaired in its biological potency.

In the present state of purification the leukocytosis-promoting factor

is capable of inducing, within several hours, a rise in the number of circulating leukocytes ranging from about 70 to 300 or even 400 per cent above the original basal level. The effect is striking when it is recalled that, within the same interval, studies on the normal variation of the leukocytic level of dogs indicate an average maximum rise of about 25 per cent.[1, 5]

As previously stated, the leukocytosis-promoting factor is not recovered from normal serum. On the other hand, the material is readily extracted from the serum of an animal with a concomitant acute pleural inflammation, thus indicating that the effective pseudoglobulin probably reaches the bone marrow via the circulating blood stream.[8] My earlier studies [1-3] have indicated that the developing leukocytosis is referable to a discharge of immature leukocytes into the circulation. This fact suggests that the leukocytosis-promoting factor acts directly on the hematopoietic tissue of the bone marrow. Is the effect merely due to an outpouring of already formed cells from the marrow, or does the substance likewise stimulate a corresponding growth or hyperplasia of myeloid elements?* The present study represents a compilation of data on the effect of the leukocytosis-promoting factor on the bone marrow of dogs.

Experimental Findings

Exudative material was obtained either from dogs or from human patients. In dogs inflammation was induced, as described in earlier communications,[9, 10] by the introduction, under nembutal anesthesia, of about 1.5 cc. of turpentine into the right pleural cavity. The leukocytosis-promoting factor was extracted as has been described. The concentration of material injected ranged from about 13 mg. to slightly over 100 mg., dissolved in physiological saline solution. In some of the experiments a single administration of the material into the circulation was employed. In other animals two or three injections were performed several hours or 1 day apart. The animal was sacrificed usually 1 or 2 days after the introduction of the leukocytosis-promoting factor. A thorough necropsy was performed. Samples of the bone marrow were obtained from the femur and from a rib. The tissue was fixed in a 10 per cent formaldehyde solution or in Zenker's fluid and acetic acid. The preparations were stained with hematoxylin and eosin or with Giemsa's stain. The bone marrow of several dogs with pleural inflammation was also studied. One animal was injected intravascu-

* Growth as used here is clearly synonymous with the term hyperplasia. It refers to an increase in the number of cellular elements in the bone marrow. This may be adequately considered as a specific growth reaction of hematopoietic tissue.

larly with the whole exudate and its marrow was subsequently fixed and stained. Two untreated dogs were sacrificed in order to obtain normal marrow. Finally, two control animals were injected with the pseudoglobulin fraction extracted from normal serum and their bone marrow was subsequently examined. The leukocytosis-promoting factor was, in several instances, recovered from exudates obtained from either the pleural or the peritoneal cavity of human patients. The material was then injected into dogs and the bone marrow subsequently studied.

The essential results of the observations appear in Table I. It is

TABLE I

The Effect of the Leukocytosis-Promoting Factor on Bone Marrow Hyperplasia

Dog no.	Type of material injected into the circulation or presence of pleural inflammation	Effect on the marrow (extent of hyperplasia indicated by number of plus signs)	Maximum increase in the number of circulating leukocytes as a result of injection of the LPF,* exudate, or serum pseudoglobulin
			per cent
7-64	LPF*	++	127.2
7-48	LPF	++	70.7
7-65	LPF	Trace to +	137.0
7-46	LPF†	++	170.4
7-49	LPF‡	+++	412.8
7-55	LPF†	+++	291.4
		Average increase in the number of leukocytes	201.6
4-43	Exudate	++	68.5
7-59	Pleural inflammation‖	++	
7-39	Pleural inflammation‖	+++	
7-62	Pleural inflammation‖	Trace to +	
7-54§	Pleural inflammation	o	Marked leukopenia, W. B. C. of 2,450
7-50	Pseudoglobulin of serum	o	30.0
7-52	Pseudoglobulin of serum	o	69.5¶
		Average increase in the number of leukocytes	49.8
7-57	Control—no injection	o	
7-41	Control—no injection	o	

* LPF = leukocytosis-promoting factor.

† Leukocytosis-promoting factor obtained from human exudate; in other experiments the material was obtained from dogs.

‡ A single injection of only 13.5 mg. of LPF made. Other animals received much larger quantities of the material, either as previously desiccated or as the fluid fraction.

§ In this animal the inflammation in the pleural cavity was accompanied by a severe leukopenia in the circulation; the bone marrow was correspondingly found to show no signs of hyperactivity.

‖ In the animals with pleural inflammation no observations were taken on the degree of ensuing leukocytosis; but previous studies[1] have indicated an average increase of 121.3 per cent in the leukocyte level of animals with similar pleural inflammation.

¶ This figure is somewhat elevated for the effect of normal serum pseudoglobulin on the leukocytic level.[7] It is to be noted, however, that the serum which was slightly hemolyzed was originally obtained from a dog (7-51) that had received a single injection of leukocytosis-promoting factor into its circulation several days previous to withdrawing the sample of blood utilized in the present dog (7-52).

clear from the data that in dogs an acute pleural inflammation *per se* induces hyperplasia of the marrow. This is characterized primarily by active proliferation of the myeloid elements as well as of the megakaryocytes. This picture is similar to that recently described by Williams [11] on the marrow of patients afflicted by a pneumonic process. The effect on the marrow is precisely the same following the injection of the whole exudate. But it is important to note that the same picture with even a more marked hyperplastic response is observed following the injection of the leukocytosis-promoting factor. The usual fat of the femoral bone marrow is now almost completely replaced by an actively hyperplastic marrow. The myeloblasts, myelocytes and megakaryocytes seem to form the predominant cell types. The effect is illustrated in Figure 1. On the other hand, either normal marrow or the marrow of animals previously injected with the pseudoglobulin recovered from normal serum fails to manifest any hyperplastic response (Fig. 2). Another evidence of the proliferative effect caused by the leukocytosis-promoting factor on the myeloid tissue of the bone marrow is revealed by observing, at least in some of the preparations, a considerable number of mitotic figures. The number of megakaryocytes, as previously mentioned, may be markedly elevated.

The strikingly high potency of the leukocytosis-promoting factor in inducing in the blood stream an average increase in the leukocyte count of 201.6 per cent (Table 1) is doubtless referable to the dissociation of the leukopenia-inducing euglobulin fraction (necrosin) from the active pseudoglobulin (leukocytosis-promoting factor) of exudates. In earlier studies [1, 3, 5] the failure to take this factor into consideration has yielded definitely weaker fractions of leukocytosis-promoting factor.

Discussion

The observations reported previously and in the present communication indicate that the leukocytosis-promoting factor recovered as a pseudoglobulin from inflammatory exudates not only offers a reasonable explanation for the mechanism of leukocytosis associated with inflammatory processes, but it is also clear that this globulin fraction induces hyperplasia or growth of myeloid cells as well as megakaryocytes in the bone marrow. This effect is readily observed both in the femoral bone marrow and in the marrow of ribs. This double potentiality of inducing an acceleration of growth and a discharge of leukocytes into the circulation may be closely linked. It is conceivable that the promptness of appearance of a leukocytosis in the circulation is conditioned by the activity of the bone marrow at the time of injec-

tion of the leukocytosis-promoting factor. A relatively aplastic marrow would probably tend to show at first a rather sluggish response to the presence of this active protein as compared to an already active marrow. This fact may perhaps account in part for the variation in time response obtained in different animals with the same fraction of the material. For instance, in one dog the leukocytosis may be elicited in about 2 hours, whereas in another animal the same material may induce a similar effect only after 12 to 24 hours. There is some suggestive evidence that both growth and maturation of the leukocytes are not only hastened in the marrow but that the maturation process may perhaps be also accelerated in the circulating leukocytes. Whether, however, this enhanced maturation actually occurs outside of the hematopoietic tissue remains to be determined with greater precision. Such studies are now in progress and will form the subject of future reports. In brief, the observations of the present experiments clearly indicate the isolation of a pseudoglobulin from inflammatory exudates which seems to have a direct and specific effect on the growth of some of the cells of the bone marrow without producing any detectable or injurious effect on other tissues. This finding may have definite clinical implications. It is known that the prognosis of numerous infectious processes depends, to some extent at least, on the level of circulating leukocytes. The injection of the leukocytosis-promoting factor, by promoting hyperplasia of the marrow and a discharge of leukocytes into the circulation, may prove to be of clinical value. It is also conceivable that this active biological substance may be of aid in agranulocytic and other aplastic conditions of the marrow. Furthermore, in view of its specific effect in hastening the growth of myeloid elements, it remains to be seen what effect the leukocytosis-promoting factor may have on the course of experimental leukemia. Such a study is now in progress.

Conclusions

An inflammatory exudate injected into the blood stream of dogs has been shown to induce a rise in the number of circulating leukocytes. The effect is due to the presence of a leukocytosis-promoting factor liberated at the site of a recent injury. This factor has been recovered as a pseudoglobulin from exudates. It penetrates into the blood stream from the site of inflammation and thus reaches the bone marrow. It induces a discharge of immature leukocytes from the marrow into the circulation. This fact offers a reasonable explanation for the mechanism of leukocytes accompanying numerous inflammatory processes.

The leukocytosis-promoting factor has also a direct and specific

growth effect on some of the cells of the bone marrow. Its injection into dogs is followed by a marked hyperplasia of the myeloid elements of the bone marrow. This is also accompanied by an augmentation in the number of megakaryocytes. The various implications of the two potentialities of the leukocytosis-promoting factor in being capable of eliciting a discharge of leukocytes into the blood stream together with a hyperplasia of granulocytic cells in the bone marrow are pointed out.

I wish to express acknowledgment to Mr. M. Kadish and Miss Carolyn Clemente for assistance in the course of this study, and to Miss E. L. Sweet for the photomicrographs.

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[*Illustrations follow*]

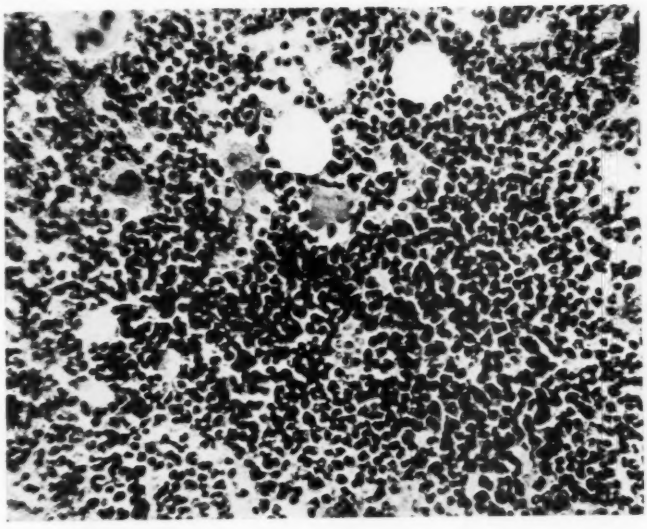
DESCRIPTION OF PLATE

PLATE 125

FIG. 1. Femoral bone marrow of dog 7–49, 2 days following a single injection of 13.5 mg. of the leukocytosis-promoting factor. The hyperplasia is striking. × 330.

FIG. 2. Femoral bone marrow of dog 7–50, 2 days following an injection of 14.5 mg. of pseudoglobulin derived from normal blood serum. There is clearly no evidence of any hyperactivity. × 330.

1



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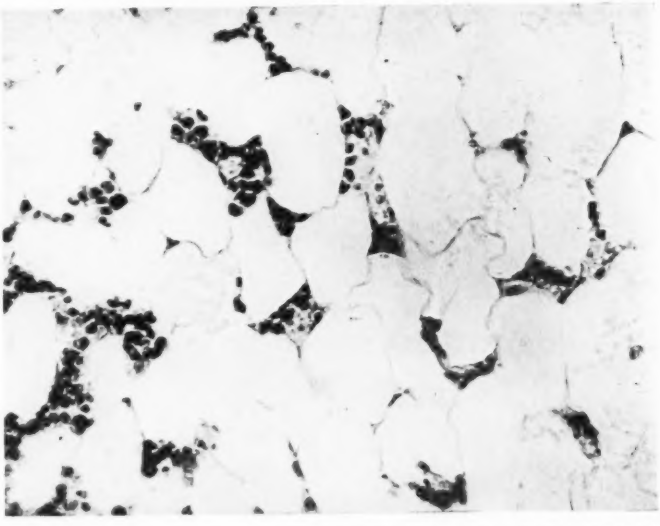

Menkin Leukocytosis-Promoting Factor and the Bone Marrow

THE ANTERIOR PITUITARY GLAND IN WOMEN WITH CARCINOMA OF THE MAMMARY GLAND, WITH REPORT OF A CASE OF CHROMOPHOBE ADENOMA*

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The purpose of the present study was to determine whether the pituitary glands of women who had carcinoma of the mammary gland showed changes which might be interpreted as hyperestrogen effects, and thus, by a new approach, to gain knowledge on the causation of human breast cancer. Our interest in such studies was aroused by a chromophobe adenoma of the pituitary gland in a woman who had mammary cancer, and by the numerous reports from experimental laboratories dealing with relationships between estrogens, the pituitary gland and breast tumors in animals.

When estrogenic hormones are injected into mice and rats, tumors of the breast are induced under certain conditions. (See reviews and recent papers by Haagensen and Randall,[1] Geschickter and Byrnes,[2] Gardner,[3] Lacassagne,[4] Cramer,[5] Greene and Brewer,[6] Allen,[7] and others.) The injection of estrogens into these animals may also produce changes in the anterior pituitary gland consisting of hyperplasia, vascular congestion and tumors (Hohlweg,[8] Wolfe,[9] Cramer and Horning,[10] Zondek,[11] McEuen, Selye and Collip,[12] Burrows,[13] Zondek,[14] Perry and Lockhead,[15] Lacassagne and Nyka,[16] Weil and Zondek,[17] Gardner,[18] and others). There is some relationship, the exact nature of which is unknown, between estrogen and the tumors in the two glands. It seems to be well established that the ovarian hormones may be one factor in the causation of breast tumors in mice.[19, 20]

In women, also, imbalance or excessive amounts of ovarian hormones have been suspected of being implicated in the causation and in the subsequent growth of breast cancer for the following reasons, among others:†

1. Mammary carcinoma has appeared in women who received estrogenic therapy in cases reported by Allaben and Owen,[21] Auchincloss and Haagensen [22] and Parsons and McCall.[23] A causal relationship has, however, not been proved by these cases. It has not been

* Received for publication, February 1, 1943.

† Complete bibliographies are not attempted. Generally only some of the recent papers, especially those having numerous references, are quoted. Also, many papers giving evidence which fails to confirm these claims are omitted. Thus the strongest possible position is presented, without rebuttal, and with the realization that the analysis is not critical.

shown that a higher percentage of women receiving estrogenic therapy develop breast cancer than of women of the same age who had no such therapy.

2. Women who have previously had oophorectomy develop less mammary gland cancer (1.5 per cent in 1,906 women) than women who have not (15.4 per cent in 1,011 women), according to Herrell.[24]

3. There are probably close relationships of estrogens to cystic mastitis, and of the latter to breast cancer (Patey,[25] Warren,[26] Taylor,[27] Logie,[28] and others).

4. The incidence of a history of menstrual irregularity is high in patients with mammary cancer (Taylor [29, 30]).

5. The menopause occurs later in women who have breast cancer than in other women (Olch,[31] Heiberg and Heiberg [32]).

6. Seventy-five per cent of the cases of breast cancer occur in the period of greatest ovarian activity or within 5 years thereof (Olch,[31] Taylor [33]).

7. There is a high coincidence of breast and uterine tumors (Taylor,[34] Meigs [35]).

8. Unmarried women have relatively more breast cancer than married women (Heiberg and Heiberg,[32] Adair,[36] Bromeis [37]).

9. The incidence of a history of faulty nursing is high in women with mammary cancer (Taylor,[33] Adair,[36] Bromeis,[37] Trout[38]).

10. The incidence of a primary carcinoma in the second breast following removal of one breast for cancer is high (Trout,[39] Kilgore [40]).

11. Improvement of premenopausal patients with mammary cancer, judged especially by bone metastases, has followed castration by surgery or sterilization by irradiation (Beatson,[41] Ahlbom,[42] Dresser,[43] Taylor,[44] Pohle [45]).

12. Breast cancer may be stimulated by pregnancy (Bromeis,[37] Trout [39]).

No one of these lines of evidence proves that estrogens are the immediate cause of human mammary gland cancer, and neither does their summation, although the sum is impressive. The accumulated observations emphasize the unusual status of these women from the hormonal point of view.

Further evidence might be found in the pituitary gland. If excessive amounts or imbalances in estrogens are factors in the causation of cancer of the human mammary gland, as well as in lower animals, perhaps the pituitary body will show changes resembling those seen in animals after injections of estrogens. Having access to human material, we have made a search for such changes.

Among thirteen pituitary bodies obtained from women who had

carcinoma of the mammary gland, one, to be described, had a large adenoma. It is possible that this represents the human counterpart of the pituitary tumors found in animals following injections of estrogens. The other twelve showed no obvious gross or microscopic deviations from normal, so that actual differential counts of the types of cells were made to detect small differences.

Methods

Twelve pituitary glands from women with mammary carcinoma were fixed, generally in formaldehyde, and embedded in paraffin, after which sections 4 μ thick were cut in the midsagittal plane. In one case horizontal sections were used. The sections were stained by a variety of methods, including Gomori's chromium hematoxylin and phloxine;[46] Mallory's phosphotungstic acid hematoxylin, and his acid fuchsin, aniline blue, orange G methods; hematoxylin and eosin; Maximow's hematoxylin, eosin, azure; and Bailey's aniline fuchsin, acid violet; and his ethyl violet, orange G methods. The latter were used because some pituitary glands, not included here, had been fixed in Regaud's solution. The differential stains on these sections were not satisfactory, and this material eventually had to be discarded. The final cell counts were made, with one exception, on sections stained by Dr. Georg Gomori by his method.

Differential cell counts were made under oil immersion. Four thousand cells from each gland were counted, 2,000 being counted independently by each of the authors. Every cell was counted which contained a nucleus, and which a pointer fixed in one ocular touched in passing across the field. The counts were begun at one capsule and continued entirely across the gland to the opposite capsule. Generally four vertical and four anteroposterior runs, evenly spaced from each other, were required by each of us.

Sections not over 5 μ thick were essential for accurate counting to avoid under-counting of those chromophobe cells which had little cytoplasm. The overlap of the heavily stained cytoplasm of adjacent chromophils could lead to counting chromophobes as chromophils. Paraffin embedding was found to be superior to celloidin because it caused a slight retraction of the cytoplasm.

As controls, differential cell counts were made by similar methods on the pituitary glands of 15 women who died of tumors located in organs other than the mammary gland. The valuable data of Rasmussen [47, 48] on normal pituitary glands supply standards and control data for work by others. However, because our material and our methods, of necessity, did not conform to his it was thought desirable

for this work to have its own controls. For instance, in both of our groups the patients did not die suddenly as did his but were ill for some time with malignant neoplasms. Our method of making counts from one midsagittal section was not ideal and it was deemed necessary to have a control group counted by the same method.

Results

Gross Appearance. With the exception of the case which had an adenoma (to be described in detail), the pituitary bodies grossly appeared to be practically normal. In those instances in which weights or measurements were recorded, they were within the normal range. One showed a tiny tumor metastasis in the posterior lobe on microscopic examination.

Percentage of Chromophobes. The chromophobes averaged 47.5 per cent in the cases with carcinoma of the mammary gland and 48.2 per cent in the controls. These data are shown in Tables I and II. In Table III it can be seen that these differences are not significant. The individual variation was great. It ranged from 35 to 57 per cent in cases of carcinoma of the breast, and from 30 to 71 per cent in the controls.

Table I

Percentage of the Different Types of Cells in Anterior Lobe of Human Hypophysis in 12 Women with Carcinoma of the Mammary Gland

Cell type	Minimum	Maximum	Median	Mean and probable error	Standard deviation	Coefficient of variation
Chromophobe	35.3	56.7	48.5	47.5 ± 1.34	7.07	14.9
Acidophil	21.9	56.0	38.4	38.9 ± 1.84	9.65	24.8
Basophil	8.4	25.9	11.8	13.6 ± 0.96	4.98	36.6

Table II

Percentage of the Different Types of Cells in Anterior Lobe of Human Hypophysis in 15 Women with Miscellaneous Tumors

Cell type	Minimum	Maximum	Median	Mean and probable error	Standard deviation	Coefficient of variation
Chromophobe	29.8	71.5	49.8	48.2 ± 1.94	11.25	23.3
Acidophil	12.0	59.4	35.1	35.4 ± 1.87	10.85	30.6
Basophil	8.8	30.1	14.8	16.4 ± 6.35	36.88	224.8

Table III

Comparison of the Mean Percentages of the Different Types of Cells in Hypophyses of Women with Carcinoma of the Mammary Gland and Those with Other Tumors

Cell type	Carcinoma of the mammary gland (12 cases)	Miscellaneous tumors	Difference	Probable error of difference	Difference / probable error of difference
Chromophobe	47.5 ± 1.34	48.2 ± 1.94	−0.7	2.35	0.2
Acidophil	38.9 ± 1.84	35.4 ± 1.87	+3.5	2.63	1.4
Basophil	13.6 ± 0.96	16.4 ± 6.35	−2.8	6.42	0.4

Percentage of Acidophils. The acidophils averaged 38.9 per cent in the breast cancer cases and 35.4 per cent in the control cancer cases. As is shown in Table III, these differences are not significant. Again, the individual variation was great, ranging from 22 to 56 and from 12 to 59 per cent, respectively, in the two groups.

Percentage of Basophils. The basophils averaged 13.6 per cent in the cases with carcinoma of the breast and 16.4 per cent in the control group. This difference is not significant, as is shown in Table III. The basophils also showed great individual variation, ranging from 8 to 26 per cent in the breast cancer cases, and from 9 to 37 per cent in the controls. Most of the counts in the control group fell between 7.4 and 18.0 per cent, but two were far greater: they were 30.1 and 37.1 per cent.

Discussion

In Table III the results of the cell counts in the two groups of patients—carcinoma of the breast and miscellaneous tumors—are compared. In the last column the ratio of the differences is given. It is less than 1.5 with respect to each type of cell, showing that the differences are not significant. The relative proportions of the different types of cells in these two groups of patients, then, may be considered to be in the same range.

There remains, however, the necessity of comparing these two groups with counts from normal women. For this purpose the best data are those of Rasmussen.[47] In Table IV the cases of carcinoma of the breast are compared with Rasmussen's normal standards, and in Table V the miscellaneous tumor cases are similarly treated. It is seen that

Table IV

Comparison of the Mean Percentages of the Different Types of Cells in Hypophyses of Women with Carcinoma of the Mammary Gland and in Normal Women

Cell type	Normal females (Rasmussen's 94 cases)	Carcinoma of the mammary gland (12 cases)	Difference	Probable error of difference	Difference / probable error of difference
Chromophobe	49.6 ± 0.47	47.5 ± 1.34	+2.1	1.43	1.5
Acidophil	43.4 ± 0.56	38.9 ± 1.84	+4.5	1.91	2.4
Basophil	7.0 ± 0.20	13.6 ± 0.96	−6.6	.98	6.7

Table V

Comparison of the Mean Percentages of the Different Types of Cells in Hypophyses of Women with Miscellaneous Kinds of Tumor and in Normal Women

Cell type	Normal females (Rasmussen's 94 cases)	Miscellaneous tumors (15 cases)	Difference	Probable error of difference	Difference / probable error of difference
Chromophobe	49.6 ± 0.47	48.2 ± 1.94	+1.4	1.99	0.7
Acidophil	43.4 ± 0.50	35.4 ± 1.87	+8.0	1.94	4.1
Basophil	7.0 ± 0.20	16.4 ± 6.35	−9.4	6.35	1.5

the results are in the same range except for one comparison in each table. These require further discussion.

In the last column in Table IV, the ratio of the differences to the probable error of these differences, with respect to basophils, is seen to be 6.7. A ratio of 4 means that there is less than one chance in one hundred that the difference is due to random sampling. This increase in basophils, then, appears to be statistically significant. This ratio is still significant (5.3) if our results are compared with those given by Rasmussen for normal women over 50 years of age. The 12 women in our group of carcinoma of the breast averaged 58.8 years of age. Eight of them were over 50 years, so that this comparison is fair.

Although the data appear to demonstrate a real increase in the basophils in women who had carcinoma of the mammary gland over those in normal women, this conclusion should not be drawn until a larger series of cases is studied. Furthermore, our sampling of the different parts of the pituitary gland was less thorough than that by Rasmussen, so that the results cannot be compared precisely because the methods were different. Even if an increase in the basophils were established, the interpretation would be uncertain because increases in this cell have been reported in numerous nonneoplastic conditions.[48, 49]

In the last column in Table V, the ratio of the differences to the probable error of these differences for acidophils is seen to be 4.1. Being over 4 this appears to be significant until the data are recalculated to compare with Rasmussen's figures for normal women over 50 years of age (average 61 years). When this is done the ratio falls to 2, a figure not statistically significant. These women with miscellaneous tumors averaged 47 years of age, and 7 of them were over 50 years. While this recalculation is not strictly justified, it casts considerable doubt on the importance of the figure of 4.1 for acidophils in Table V. The conclusion is that a reduction of acidophils has not been demonstrated beyond doubt in women with various types of tumors.

In mice, mammary gland tumors may be caused by several different factors, according to Bittner.[19, 20] Although the causation of carcinoma of the mammary gland in women is less well known, it is possible that different combinations of factors might be operating in different cases. For example, the rôle of estrogenic hormones might be more important in premenopausal mammary gland cancer than in that which begins after the menopause. If this is true, the pituitary glands might be different in the various age groups. When our data were examined from this point of view no differences were revealed. The proportions

of the three types of cells were approximately the same in the 4 women who were under 50 years of age as in the 8 who were over that age.

Data on the condition of the pituitary gland in women with carcinoma of the breast were not found in the literature except in so far as they could be culled from general papers. No reports of differential counts were found although many changes, unsupported by evidence, were reported. The pituitary gland did not appear to be enlarged in the case of carcinoma of the breast in Wyeth's [50] tables. The average weight for his 8 cases was 0.878 gm. as contrasted with an average of 1.050 gm. for 3 women with carcinoma of the uterus, and an average of 0.811 gm. for 28 women with tumors of other types.

There is agreement among all observers that the chromophobes are the cells which are responsible for the enlargement of the pituitary gland in animals following estrogenic therapy.[17, 51] On the basis of 12 cases reported here there is no evidence that the chromophobes are increased in women with carcinoma of the breast. The failure to demonstrate changes in the chromophobe cells does not exclude a hormonal factor in the causation of these mammary gland tumors because it is not known that the human being reacts in the same way as do rodents. Furthermore, changes in the pituitary gland are not found constantly in animals with estrogen-induced mammary gland tumors. Unfortunately, in the 3 human cases of carcinoma of the breast, described by others, which followed estrogenic therapy the condition of the pituitary gland was not stated. Such observations should be made if additional cases are discovered. Neither have changes in the pituitary gland been described in women with granulosa cell tumors of the ovary.

Case of Chromophobe Adenoma of the Pituitary Gland

Although there were no visible enlargements and no changes in the chromophobe cells in the hypophyses of 12 women who had carcinoma of the mammary gland, as reported in the preceding section, a 13th case with this disease had a chromophobe adenoma. The question arises as to whether this is the human counterpart of the tumors seen in animals or whether it was coincidence.

Besides the changes in the pituitary gland which follow the injection of estrogens in animals, several observations related to the natural disease are of interest. Thus, Gardner, Strong and Smith [52] described, in a mouse, the spontaneous occurrence of tumors in the mammary gland, the pituitary body and the ovaries. Wolfe, Bryan and Wright [53] found that chromophobe cells were generally more abundant in rats which had mammary tumors.

Report of Case

Mrs. R. P., white, aged 42, entered the University of Chicago Clinics on September 14, 1931, for the removal of a breast tumor which had been present for 4 months. The breast, containing a carcinoma, was amputated and the axillary lymph nodes, also containing carcinoma, were resected. The patient died of pneumonia 6 weeks after operation. Necropsy (by Dr. E. M. Humphreys) revealed small but widespread carcinoma metastases in the pleura, lungs, liver, adrenals, brain, and lymph nodes of the thorax and abdomen.

That part of the record which was possibly related to the pituitary gland was as follows: Her menses began at 12 years. They were painless, regular at 28 days, and lasted for 2 days with a moderate flow. She had married at 20 years of age, and had been pregnant six times, giving birth to six living children. In 1925, when she was 36 years old, she was knocked unconscious in an automobile accident, following which the menses stopped permanently. Two years later she was hospitalized for 3 months because of a polyarthritis. The only manifestations possibly related to enlargement of the pituitary gland were attacks of vomiting, and mental confusion.

At necropsy the pituitary gland was found to be enlarged to about three times its normal size. The sella turcica was enlarged and the clinoid processes were eroded. The gland was still encapsulated. It pressed upward against the floor of the third ventricle and compressed the optic chiasm and the optic nerves.

Microscopic examination of this tumor disclosed that it was composed of masses of cells with scant cytoplasm and fairly small oval nuclei. There were no mitotic figures. The tumor had little stroma. Special stains failed to reveal any cytoplasmic granules. The structure was compatible with the diagnosis of the small cell type of chromophobe adenoma.

There were no remarkable changes in the adrenals, ovaries, uterus, thyroid, thymus, pancreas, or remaining mammary gland.

Comment

It is difficult to make final interpretation of this case at the present time. The coexistence of an adenoma of the pituitary gland and carcinoma of the mammary gland may have been without significance, since the incidence of pituitary adenomas in routine autopsies has been stated to be 10 per cent.[54-57] While true adenomas are occasionally encountered as incidental findings, this high figure is open to some question, because of the criteria used as to what constitutes an adenoma. In many pituitary glands areas are found which, at first glance, appear to be composed of one type of cell but more careful study reveals that there is an admixture of cells of other types.

It is impossible to attribute with assurance either the mammary gland carcinoma or the pituitary gland adenoma to an increased amount of estrogen because menstruation had stopped 6 years before

the tumors were recognized, and because the uterus showed no hyperplasia. Although elaborate theoretical explanations could be devised, they would not prove the points at issue.

A follow-up on persons who survived operations for chromophobe adenomas from the point of view of the incidence of subsequent development of carcinoma of the mammary gland has not been made on a large series of patients to our knowledge. Neither have we found a record of a high incidence of pituitary gland adenomas in cases of cancer of the breast. The incidence of subclinical pituitary gland adenomas is about the same in the two sexes, which is unlike the sex ratio of breast cancer. The incidence of adenomas of the pituitary body has been stated to be increased in cases of adenoma of the prostate gland, carcinoma of the pancreas, carcinoma of the rectum, and in carcinoma of the bronchus.[57]

Summary

Differential cell counts were made on the anterior lobes of the pituitary glands obtained from 12 women who had carcinoma of the mammary gland, and, as controls, on pituitary glands from 15 women who had tumors of other types. There were no important differences in the percentages of chromophobes, acidophils or basophils in these two groups.

When these cell counts were compared with those given by Rasmussen[47,48] for normal women, the chromophobes and the acidophils were within normal limits while the basophils were increased. Since the size of the group was small, and the sampling of all parts of the pituitary glands was not thorough, this difference should not be accepted as final.

Although the chromophobe cells were not increased in 12 cases of carcinoma of the mammary gland, in a 13th case an adenoma, of chromophobe cell type, was found.

Thus, there was no evidence found in the 12 pituitary glands for hyperestrogen effects, although it is possible that the human pituitary gland does not respond to estrogens in the same way as does that of rodents. The adenoma, however, might possibly be considered as evidence for a hyperestrogen effect, although proof of this cannot be offered.

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DECIDUAL REACTIONS IN FALLOPIAN TUBES

Histologic Study of Tubal Segments from 144 Post-partum Sterilizations *

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This report is based upon histologic examination of excised tubal segments from 144 consecutive post-partum sterilizations done at the Kapiolani Maternity and Gynecological Hospital during the 3-year period from 1940 to 1942 inclusive. It was prompted by the frequent observation, during the past 2 years, of a decidual reaction in such tubal segments. Consequently, all tubes removed at post-partum sterilizations during 1942 have been studied more carefully than is the usual custom with such specimens. In most cases sections were prepared at intervals through each tubal segment and in a number of instances complete serial sections were made. In addition, all sections of post-partum tubes removed during the preceding 2-year period were reviewed, although in most of these cases only one microscopic section through each tubal segment was available for examination. The tubal segments varied in length from 1 to 2 cm. and in all but 2 cases comprised the proximal (isthmian) portions.

One or both tubal segments from 17 (12 per cent) of these 144 cases exhibited decidual formation of varying extent and location. These cases are analyzed in Table I.

One-half of the patients making up this group of 144 cases were Hawaiian or part Hawaiian in nationality. The majority of the remainder were Japanese, Chinese, and Filipinas, and there were relatively few Caucasians. Eleven (64 per cent) of the 17 cases showing a tubal decidual reaction belonged to the Hawaiian or part Hawaiian group and there were only 3 Caucasians (2 of them Portuguese) in the remaining 6. The significance of this finding will be considered later.

The elapsed time in hours between delivery and operation varied from 0 (cesarean section) to 65. There was no constant correlation between the length of time between delivery and operation and the presence of decidual tissue or the amount of decidual cell involution.

*Read before the Staff Meeting of The Clinic, January 9, 1943.
Received for publication, February 15, 1943.

TABLE I

Analysis of 17 Post-partum Sterilizations in which Decidual Change was Found in the Excised Tubal Segments

Case no.	Race	Age	Para	Hours post-partum	Number of sections studied	Location of decidua	Amount of decidua	Involution	Serosal mesothelium
1	Pt. Hwn.	28	4	28	Serial	Mucosal and serosal	Great	Advanced	Cuboidal
2	Cauc.	26	5	20	1	Mucosal	Slight	Slight	Flat
3	Pt. Hwn.	26	4	60	4	Mucosal and serosal	Slight	Slight	Cuboidal
4	Pt. Hwn.	28	3	0	1	Serosal	Slight	None	Flat
5	Hwn.	24	4	47	1	Mucosal	Slight	None	Flat
6	Hwn.	33	7	44	1	Mucosal	Moderate	Moderate	Flat
7	Cauc.	30	2	57	2	Mucosal	Moderate	Moderate	Cuboidal to columnar
8	Hwn.	33	4	12	Serial	Mucosal	Great	Slight	Cuboidal
9	Cauc.	30	2	0	1	Serosal	Slight	None	Cuboidal
10	Pt. Hwn.	25	4	58	1	Mucosal	Slight	Advanced	Flat
11	Pt. Hwn.	27	4	32	1	Mucosal	Moderate	Slight	Flat
12	Pt. Hwn.	34	2	0	Serial	Mucosal	Slight	None	Cuboidal
13	Jap.	38	6	8	1	Serosal	Moderate	Moderate	Cuboidal
14	Jap.	38	2	27	Serial	Mucosal	Great	Advanced	Cuboidal
15	Pt. Hwn.	25	4	20	1	Serosal	Slight	Moderate	Cuboidal to columnar
16	Jap.	28	4	65	100 μ apart	Serosal	Moderate	Moderate	Cuboidal with invaginations
17	Hwn.	32	10	21	100 μ apart	Mucosal and serosal	Moderate	Slight	Cuboidal

Hwn. = Hawaiian
Pt. Hwn. = Part Hawaiian
Jap. = Japanese
Cauc. = Caucasian (including Portuguese)

Location of Decidua

In 9 cases the decidual tissue occupied the mucosal folds, in 5 it was subserosal in location and in the remaining 3 it was found in both locations. The involved mucosal folds were swollen and distended by the decidual cells, and the covering tubal epithelium was, without exception, greatly flattened but always recognizable as a distinct layer (Figs. 2 and 4). The tips or free margins of the rugae showed the greatest change; toward the bases of the folds decidual cells were rarely found. Where the process was extensive the rugae appeared as pedunculated spherical masses attached to the wall by narrow pedicles (Fig. 1). Where involution was slight the decidual cells were well preserved structurally and presented the characteristic mosaic pattern seen in the endometrium (Fig. 4). The cells, however, tended to vary somewhat in size and shape and many of them were elongated and spindle-shaped.

The serosal decidua in general was less well developed than that in the mucosa, and there was even greater variation in the size and shape of the cells. In only one instance (case 1) was there a continuous mantle of cells surrounding the tube. In the remaining cases the cells existed in the form of small accumulations just beneath the mesothelium. The patches of decidua were most numerous in the region of the mesosalpinx and often existed in the form of small evaginations covered by flat serosal mesothelium (Fig. 5).

Amount of Decidua

In cases 1, 8 and 14 the decidual tissue was abundant, practically all of the mucosal folds in both segments showing this change, and serial sections in these 3 cases showed the decidual tissue to extend longitudinally throughout the entire length of the tubal segment (Fig. 1). In 6 cases the decidual formation was moderate in amount (less than one-half of the mucosal folds involved; two to four patches of decidua just beneath the mesothelium in those with serosal involvement). In 8 cases only slight decidual change was present. These exhibited from one or two cells up to one or two groups of cells either in the tip of a mucosal fold or in a patch beneath the serosa (Fig. 3).

Decidual Cell Involution

In 9 cases there was no, or at most very slight, decidual cell involution; moderate or advanced involution was present in the remaining 8. Study of the latter group of cases yielded interesting data on decidual cell involution. The initial stage in this process is

the appearance of a large round vacuole in the center of the cell with displacement of the nucleus toward the periphery (Fig. 5). This is followed by additional large vacuoles resulting in complete disappearance of the cytoplasm and further peripheral displacement of the nucleus. At this stage the cell is large and appears as a ring form not unlike the signet ring cell of mucoid adenocarcinoma (Fig. 6). The nucleus is elongated or band-like and is located far to the periphery at one margin of the cell. The nucleus then disappears and the cell shrinks and becomes irregular, forming a faintly staining small teardrop or crescent-shaped structure which soon vanishes.

The end result of this process is the formation of a cyst or cysts at the decidual cell site. As each cell involutes it leaves behind the space which it formerly occupied. Thus in those cases showing involution, the decidual cells were loosely arranged, each separated from its fellow by a clear space, and the remaining cells were in various stages of the degenerative process already outlined (Fig. 2). In several locations this process was so extreme that the bulbous mucosal fold had been largely converted into a cyst in which only a few decidual cell remnants could be identified. A sprinkling of lymphocytes was usually present in the involuting areas.

Serosal Mesothelium

In 11 of the 17 cases the serosal mesothelium was hyperplastic in nature and cuboidal to columnar in appearance. The cells had undergone peculiar rounded swelling due to increase in the cytoplasm (Figs. 5 and 7). The nuclei were round or oval and were located centrally. In 1 case invaginations into the underlying tissue were observed. Serosal hyperplasia was also found in many of the cases which failed to exhibit decidual tissue. The serosal mesothelium over the patches of decidua could usually be recognized as an intact and separate layer although it was greatly flattened in these locations (Fig. 5).

Historical Aspects

It has long been known that decidual reactions occur in many locations remote from the uterus. Novak [1] mentioned that such reactions have been described on the surface of the uterus, the anterior surface of the rectum, the floor of the cul-de-sac, the omentum, the ovary, the appendix, the cervix, the vagina and the peritoneum of the small intestine. Weller,[2] in 1935, reviewed the literature on this subject and discussed the significance of the ectopic decidual reaction in endometriosis. He stated that ectopic decidua on the pelvic peri-

toneum was first described by Walker [3] in 1887 and the observation was soon confirmed by other workers. It is interesting to note that eleven of Weller's twelve references to the subject are in the German literature.

Ectopic decidual formation probably occurs more often than is generally appreciated. For example, Williams [4] found ectopic decidual tissue in every fourth or fifth uterus removed at operation or autopsy at the end of pregnancy, an incidence of 20 to 25 per cent. Similarly, Hofbauer [5] demonstrated ectopic decidua on the posterior aspect of the pregnant uterus in 15 of 23 specimens examined.

The ability of the tube to undergo decidual reaction was for many years a matter of controversy but it is generally recognized at the present time that such reactions do occur. Most authors agree that the tubal decidual reaction is slight in amount and patchy in location, approaching in no way the extensive decidual membrane formed in the uterus. The great majority of observations have been in connection with tubal pregnancy, particularly in reference to decidual formation at the implantation site. Williams [4] stated that "occasionally, in uterine pregnancy, decidual cells may develop in the stroma of the tubal mucosa, but they never lead to the formation of a continuous membrane as in the uterus. Such observations are of extreme rarity but I have made them in several instances."

Kline [6] studied 74 cases of extra-uterine pregnancy and presented evidence indicating that a decidual reaction of greater or less extent occurred constantly at the site of implantation. However, he observed a distant decidual reaction in the tube in only 3 of 51 cases examined. Kline thought that the decidua at the implantation site involuted rapidly with degeneration of the chorionic villi, whereas distant decidua in the tubes, uterus, or elsewhere sometimes persisted after degeneration of the trophoblast and complete involution of the local decidua. The observations in the present series of cases would seem to confirm this statement.

According to Frankel and Schenck,[7] decidual tissue was described in tubal pregnancy by Webster [8] in 1897. These authors further stated that Osiakina and Schmatok,[9] in 1933, found a decidual reaction in the tube in 21 per cent of a series of 21 tubal pregnancies independent of the localization of the implanted ovum.

Geist and Matus [10] stated: "It also seems likely that decidua is much more common in the tube than has been hitherto believed and that study of sufficient material may demonstrate this." These workers found decidual tissue most often in the connective tissue septa of the villi and believed that it "persisted for a much longer period of time

[than uterine decidua] because the involutionary process in the tube at this depth is decidedly slower than in the uterus."

Although a complete historical review was not possible, no report similar to the present one was found. In general there appears to have been surprisingly little written on the subject of ectopic decidual reactions.

Discussion

The demonstration of decidual tissue in 12 per cent of 144 tubes removed post-partum is of theoretic importance in the etiology of tubal pregnancy. It is generally taught that the most important etiologic factors are mechanical ones. This study indicates that unusual receptivity of the tubal mucosa to the fertilized ovum may play a more important rôle than is generally believed. This idea has been advanced particularly by Frankel and Schenck,[7] who believe that ectopic pregnancy results from implantation of the fertilized ovum on a receptive nidus of aberrant endometrium in the tubal mucosa or elsewhere. They believe that such a nidus of uterine mucosa is a prerequisite for the implantation and development of the ovum in a site remote from the uterus. This theory has much to recommend it but, as yet, proof is lacking. In not a single instance in the present study was aberrant uterine mucosa found. The extensive formation of decidua encountered in at least 3 of our cases suggests, nevertheless, that receptivity of the tubal mucosa may be an important factor in the causation of tubal gestation, entirely apart from the presence of actual uterine mucosa.

This statement is of particular significance in view of the high incidence of ectopic gestations in Hawaii. Schattenburg [11] has recently investigated this question. He found that during the 5-year period from 1936 to 1940 inclusive there were 4,964 deliveries and 75 ectopic pregnancies at The Queen's Hospital. The total number of tubal pregnancies thus comprised 1.5 per cent of the deliveries, a much higher figure than the ones usually given. For example, Schumann [12] stated that 1 in every 303 pregnancies is extra-uterine, an incidence of only 0.303 per cent. Salpingitis, considered to be one of the chief predisposing factors in the causation of ectopic gestation, is relatively infrequent in Hawaii. Perhaps this is a reflection of the exceptionally low venereal disease rate which exists here.[13] May not the high incidence of ectopic gestations in Hawaii result from unusual hormonal "responsiveness" of the tubal mucosa, perhaps due to local climatic or racial factors? The present report suggests this since 11 of the 17 patients evincing a tubal decidual reaction were Hawaiians or

part Hawaiians and there were only 3 Caucasians in the remaining 6.

Be that as it may, a knowledge of the various stages in the involution of ectopic decidual cells is of practical importance to the pathologist. Interpretation is difficult when the peculiar ring forms associated with this process are observed for the first time. The degenerative process, when present, was identical in both the mucosal and serosal decidua and we have likewise found it in 3 of 5 cases of endometriosis associated with pregnancy. It may also be observed in uterine decidua, although the changes are not so well defined since the factor of inflammation is almost invariably present. Hofbauer,[5] in his excellent paper published in 1929, noted the variation in size and shape of ectopic decidual cells and observed that vacuoles in the cytoplasm were common. We feel sure that cytoplasmic vacuolization is an initial stage in decidual cell involution.

The decidual cells arise from mesenchymal cells in the stroma of the folds and from similar cells beneath the serosal mesothelium. This process was described by Hofbauer[5] who believed that such multipotent mesenchymal cells could give rise, not only to decidual cells, but also to unstriated muscle fibers. There was not the slightest suggestion in any of our cases that the decidual cells were derived from the tubal epithelium; the latter was always flattened by pressure. It is equally unlikely that the cells of the serosal mesothelium ever give rise to decidua. The serosal mesothelium could usually be recognized as a distinct although greatly flattened layer over the patches of decidua; elsewhere it was often hyperplastic and appeared cuboidal, even columnar, in cross section.

Summary

Decidual tissue was demonstrated in one or both tubal segments from 17 of 144 post-partum sterilizations, an incidence of 12 per cent.

In 9 cases the mucosal folds were involved, in 5 there were patches of decidua beneath the serosa, and in 3 decidua was present in both locations.

The decidual tissue varied in amount from one or two cells in the tip of a mucosal fold in 2 cases to masses of cells in all of the mucosal folds in 3. Serial sections in the latter 3 cases showed the decidual tissue to extend longitudinally the entire length of the tubal segment.

In 8 of the 17 cases decidual cell involution of varying degree was present. This process is characterized by vacuolization of the cytoplasm and flattening and peripheral displacement of the nucleus. The cytoplasm then disappears entirely, resulting in peculiar irregular ring forms somewhat resembling the signet ring cells of mucoid ad-

enocarcinoma. The final stage is represented by faintly staining, small, irregular shadow or ghost forms.

The decidual cells arise from mesenchymal cells in the stroma of the folds and from similar cells beneath the serosa. The tubal epithelium over the areas of decidual tissue formation is greatly flattened by pressure; elsewhere it is normal. The serosal mesothelium covering the patches of serosal decidua is similarly flattened; in other locations it is often hyperplastic, appearing cuboidal or even columnar in nature.

Conclusions

1. This study indicates that decidual reactions in fallopian tubes may occur more often than is generally believed.

2. The fact that 67 per cent of the patients showing a tubal decidual reaction were Hawaiians or part Hawaiians, together with our inability to find a similar report in the literature, suggests, however, that the phenomenon may be a purely local one, perhaps dependent upon racial or climatic factors.

3. It is further suggested that it may have an etiologic bearing upon the high incidence of ectopic gestation in Hawaii.

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[*Illustrations follow*]

DESCRIPTION OF PLATES

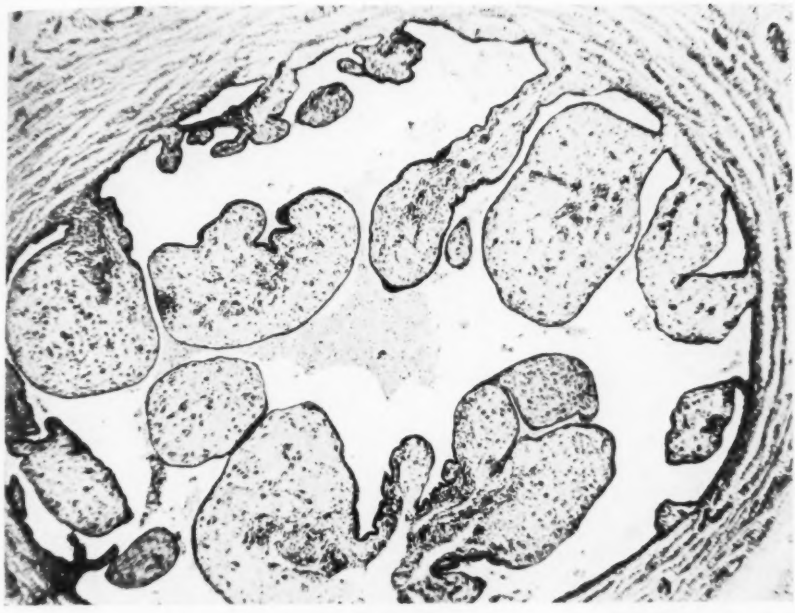
PLATE 126

FIG. 1. Case 1. Every mucosal fold is distended by decidual cells. Advanced involution is present, leaving most of the folds partially cystic. Hematoxylin and eosin stain. × 40.

FIG. 2. Case 1. A distended mucosal fold attached to the wall by a relatively narrow pedicle. The tubal epithelium is flattened by compression. Extensive involution is present, leaving the fold partially cystic. The remaining decidual cells are in various stages of degeneration and vacuolated cells and ring forms are evident. Hematoxylin and eosin stain. × 130.

FIG. 3. Case 5. Two structurally intact decidual cells are present in the tip of a mucosal fold. The covering epithelium is flattened. Hematoxylin and eosin stain. × 300.

1



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Tilden and Winstedt Decidual Reactions in Fallopian Tubes

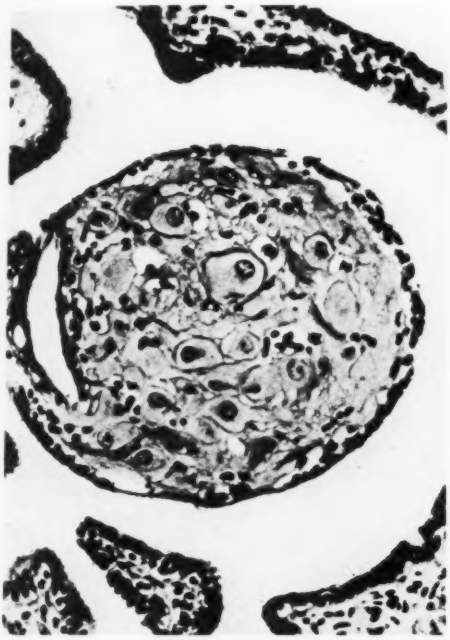
PLATE 127

FIG. 4. Case 8. A mucosal fold distended by a mass of structurally well preserved decidual cells. The cells vary somewhat in size and shape and the covering tubal epithelium is flattened. Hematoxylin and eosin stain. × 300.

FIG. 5. Case 16. A group of decidual cells in a peritoneal evagination near the mesosalpinx. Here are seen vacuolated cells, ring forms and an intact but greatly flattened covering layer of serosal mesothelium. The structure below is cuboidal mesothelium which has become detached from the underlying tissue. Hematoxylin and eosin stain. × 300.

FIG. 6. Case 1. Involuting decidual cells. Most of them exist as peculiar, somewhat irregular ring forms. Hematoxylin and eosin stain. × 300.

FIG. 7. Case 12. The serosal mesothelium is cuboidal in appearance, the cells rounded due to cytoplasmic increase, and the nuclei are located centrally. No decidual cells are present. Hematoxylin and eosin stain. × 300.



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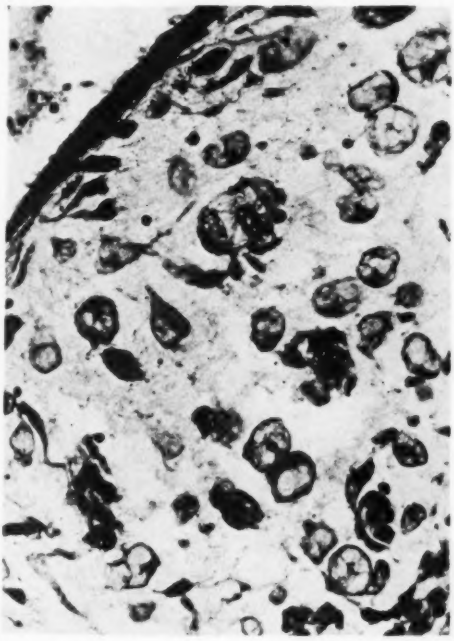

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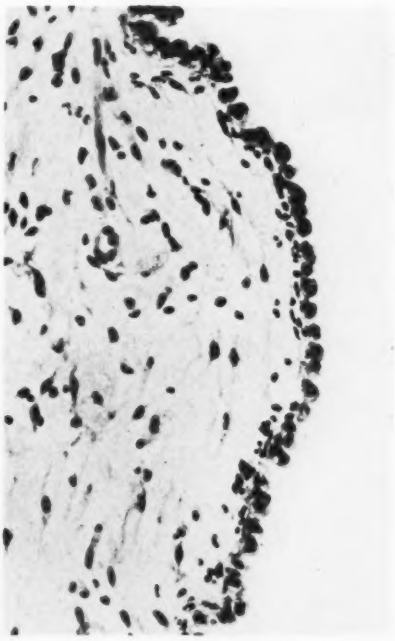

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is owned and controlled by the American Association of Pathologists and Bacteriologists and is edited by a Board appointed by the Council of the Association. It is devoted to the prompt publication of original observations and investigations in the fields of Pathology and Bacteriology, especially Morphological Pathology, and is published bimonthly to form one volume of at least six hundred pages a year. Title pages and index for the current volume are included in the November issue.

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PRINTED AT THE ANN ARBOR PRESS, ANN ARBOR, MICHIGAN, U.S.A.